TO BE “PAVAROTTI” IN A CROWDED CONCERT HALL? SONG COMPETITION BETWEEN BUSHCRICKET MALES IN NATURAL CHORUSES.

DISSERTATION

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"Anybody who has ever had to judge an athletic, music, or beauty contest knows how crucial it is for the athletes to compete under highly regulated and calibrated conditions, for the musicians to play under similar conditions, or for the beauty contestants to appear in similar clothing, precisely in order to tell the fine differences between them and select the best of the best".

Amotz, and Avishag Zahavi - *The handicap principle: A missing piece of Darwin's puzzle*, 1999

Abstract

Since the presentation of Darwin's sexual selection theory, it has become clear that selected traits of male orthopteran insects evolve in response to female choice (intersexual selection) and male-male competition (intra-sexual selection). Grasshoppers, crickets and tettigoniids have served as excellent model organisms for studying female sexual selection and acoustic signalling behaviour in males. This is especially true of bushcrickets (Orthoptera: Tettigoniidae), generally only the males of which produce sounds, mainly to attract conspecific females and to compete against rivals. During mating, males transfer a spermatophore to the female, which receives a direct benefit in terms of nutritional substances. Generally, females choose heavier males, receiving larger nuptial gifts, and detecting the best donator through his honest acoustic cues. However, too little is known of orthopterans to test how sexually selected factors may cause the plasticity of the males' acoustic features. The production of sexually selected acoustic traits is energetically demanding and can impose severe costs for the male signaller. Females try to select the best partner through his sound traits, which transfer information about his genetic quality. Sound performance can vary between individuals of different body conditions, leading to relevant consequences for the outcome of sexual selection.

This thesis aims to investigate the effect of sexual selection on secondary male traits, such as the size of sound production organs and the structure of acoustic signals. The focus is on the factors that internally determine the quality of the male signalling individual, such as his body mass, as well as external factors such as population density and composition. To fulfil the aims, two congeneric bushcrickets species of the genus *Poecilimon* were used*: Poecilimon ampliatus* and *Poecilimon v. veluchianus*. Males of different body conditions, in terms of body mass, were tested under different competition levels, both in the laboratory and in the field. Their temporal signal patterns, such as verse rate and duration, syllable rate, and duty cycle were measured and compared.

The results showed that in *P. ampliatus*, sexual selection plays a crucial role in determining the size of morphological structures that are responsible for producing female-preferred acoustic signals. Heavier and larger males had larger sound-producing organs, and specifically, they possessed larger wings, and longer stridulatory files, with a reduced density and number of teeth. The scaling relation found between wing area and femur length highlighted the positive effect of body size on this organ.

A further effect of sexual selection pressure is highlighted during male competition. Males of different body masses present discrepancies in their ability to vary their signal production and to show a plastic signal response. The main findings suggest that the modification of the acoustic social environment might induce plastic behaviour in signalling males, depending on their body mass. In both tested species, only heavy males were able to adjust their acoustic behaviour depending on the social context during competition. In contrast, light males did not vary their signal features, which remained almost constant through different competition levels. Heavier males of *P. ampliatus* signalled with significantly longer verses and a higher duty cycle than lighter males under high and

middle competition levels. When males were recorded in isolation, the acoustic activity of light and heavy males did not significantly differ, thus confirming the importance of testing signalling males during acoustic contest. However, under stronger competition pressure, other results are found in males of *P. v. veluchianus.* Heavy males tested under the highest level of competition (against two heavy rivals), signalled with shorter verses and a reduced number of syllables than when competing against one rival. In the latter case, the amount of the focal male's signal production was independent of the rival's body mass. Furthermore, when competing with light rivals, focal heavy males signalled more when facing two rivals than when facing only one.

In sum, the morphological findings indicate that positive selection on sound-producing structures is in line with the female preference for heavier males in mate choice experiments and hence, they reveal how male body mass is an honest cue for the size of sound-producing organs. The results from the acoustic data reflect the crucial relevance of combining different sources of environmental factors. In addition, they show how males pay attention to acoustic cues produced by neighbouring rivals, adjusting their signal production coherently with the strength of the competition and their individual energetic status. Bushcricket males in both species, *P. ampliatus* and *P. v. veluchianus,* present an inter-individual variation in the capacity to adjust their signal production shaped by factors both internal and external to the signalling males.

A future endeavour will be to study how males in nature evolve condition dependent signalling and alternative behavioural strategies.

Zusammenfassung

Seit der Veröffentlichung der Theorie der sexuellen Selektion durch Darwin ist es bekannt, dass die Entwicklung männlicher Genitalien durch Selektionsmechanismen wie intersexuelle (Weibchenwahl) und intrasexuelle Selektion (Konkurrenz zwischen Männchen) beeinflusst werden. Feldheuschrecken, Grillen und Laubheuschrecken haben sich als hervorragende Modellorganismen für die Untersuchung der weiblichen sexuellen Selektion und des akustischen Signalverhaltens erwiesen. Dies gilt insbesondere für Laubheuschrecken (Orthoptera: Tettigoniidae), bei denen von Männchen erzeugte Laute für das Anlocken potenzieller Partner und zur Abschreckung von Konkurrenten genutzt werden. Bei vielen Laubheuschreckenarten produzieren nur Männchen Gesänge, hauptsächlich um arteigene Weibchen anzulocken und sich mit Rivalen zu messen. Während der Paarung übertragen die Männchen eine Spermatophore, die von den Weibchen verzehrt wird. Im Allgemeinen wählen Weibchen schwerere Männchen, welche größere Brautgeschenke übertragen und erkennen diese Männchen an ihren ehrlichen akustischen Signalen.

Allerdings wurde bei Orthopteren bisher kaum untersucht, wie sexuell ausgewählte Faktoren die Plastizität der akustischen Merkmale der Männchen beeinflussen können. Die Produktion -durch sexuelle Selektion ausgewählter- akustischer Signale ist energetisch anspruchsvoll und kann für den männlichen Sender erhebliche Kosten verursachen. Weibchen versuchen, den besten Partner über seine Gesangsstruktur auszuwählen, die Informationen über seine genetische Qualität vermitteln. Die Gesangsleistung kann aufgrund der unterschiedlichen Körperqualität von Männchen zu Männchen variieren, was zu relevanten Konsequenzen für das Ergebnis der sexuellen Selektion führt.

In dieser Arbeit soll die Auswirkung sexueller Selektion auf sekundäre Merkmale der Männchen untersucht werden, wie z. B. die Größe der Organe zur Schallproduktion und die Struktur akustischer Signale. Der Fokus liegt sowohl auf den Faktoren, die intern die Qualität des signalisierenden Individuums bestimmen wie die Körpermasse des Männchens als auch auf externen Faktoren wie der Populationsdichte. Um diese Ziele zu erreichen, wurden zwei Laubheuschreckenarten der Gattung *Poecilimon* verwendet, *Poecilimon ampliatus* und *Poecilimon v. veluchianus*. Männchen mit unterschiedlicher Körpermasse wurden sowohl im Labor als auch im Feld unter verschiedenen Wettbewerbsbedingungen getestet. Ihr zeitliches Signalmuster wie Versrate und -dauer, Silbenrate und "duty cycle" wurde gemessen und verglichen.

Die Ergebnisse zeigten, dass bei *P. ampliatus* die sexuelle Selektion eine entscheidende Rolle bei der Bestimmung der Größe von morphologischen Strukturen spielt, die zur Erzeugung der von Weibchen bevorzugten akustischen Signale verwendet werden. Schwere und größere Männchen hatten größere lautproduzierende Organe und besaßen vor allem größere Flügel und längere stridulatorische Felder mit einer verringerten Zähnchendichte. Die hyperallometrische Beziehung zwischen Flügelbereich und Körpergröße unterstrich dabei die positive Auswirkung der sexuellen Selektion auf dieses Organ: seine Größe nimmt um mehr als das Doppelte der Femurlänge zu.

Ein weiterer Effekt des sexuellen Selektionsdrucks zeigte sich durch die Beobachtung der Männchen während des Wettbewerbs mit arteigenen Konkurrenten. Männchen mit verschiedenen Körpermassen zeigten Differenzen in ihrer Fähigkeit, ihre Signalproduktion zu variieren und eine plastische Signalantwort zu produzieren. Die Hauptergebnisse deuten darauf hin, dass die Veränderung des akustischen Umfelds in einer Population in Abhängigkeit von der Körpermasse das männliche Signalverhalten verändern kann.

Bei beiden getesteten Arten konnten nur schwere Männchen ihr akustisches Verhalten während des Wettkampfs an den Konkurrenzdruck anpassen. Im Gegensatz dazu variierten leichte Männchen ihre Signalmerkmale nicht, was bei unterschiedlichen Wettbewerbsniveaus nahezu konstant blieb. Schwere Männchen von *P. ampliatus* signalisierten bei hohem und mittlerem Konkurrenzniveau mit deutlich längeren Versen und höherem duty cycle als leichtere Männchen. Wurden die Gesänge von isolierten Männchen aufgezeichnet, unterschieden sich die akustischen Aktivitäten von leichten und schweren Männchen nicht signifikant. Unter stärkerem Wettbewerbsdruck veränderten sich die Ergebnisse des Verhaltens von *P. v. veluchianus* : Schwere Männchen, die unter dem höchsten Konkurrenzdruck (gegen zwei starke Rivalen) getestet wurden, produzierten kürzere Verse mit einer geringeren Anzahl von Silben, im Vergleich zum Test gegen ein einzelnes Männchen, unabhängig von dessen Körpermasse. Im Wettbewerb mit leichten Konkurrenten signalisierten schwere Männchen mehr, wenn sie gegen zwei Konkurrenten und weniger, wenn sie gegen ein Männchen antraten.

Zusammenfassend zeigen die morphologischen Befunde, dass die positive Selektion auf klangproduzierenden Strukturen mit der - in Partnerwahlversuchen ermittelten- Präferenz von Weibchen für schwerere Männchen übereinstimmt und somit, dass die Körpermasse der Männchen ein ehrlicher Hinweis auf die Größe der schallerzeugenden Organe ist. Die Ergebnisse der akustischen Daten betonen die entscheidende Relevanz der Kombination verschiedener Quellen von Umweltfaktoren. Sie zeigen, wie Männchen auf akustische Signale reagieren, die von benachbarten Konkurrenten erzeugt werden, indem sie ihre Signalerzeugung kohärent an die Stärke der Konkurrenz und ihren persönlichen energetischen Status anpassen. Laubheuschrecken-Männchen der beiden Arten *P. ampliatus* und *P. v. veluchianus* zeigen eine interindividuelle Variation in der Fähigkeit, ihre Signalproduktion anzupassen, die sowohl durch interne als auch externe Faktoren der signalisierenden Männchen beeinflusst wird.

In zukünftigen Untersuchungen könnte der Frage nachgegangen werden wie Männchen in der Natur von ihrer Kondition abhängige Signale und alternative Verhaltensstrategien entwickeln.

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Chapter 1. General Introduction

1.1. Scientific background

1.1.1 Sexual selection theory

When Darwin presented his second book on sexual selection theory in 1871, it became clear that the evolution of secondary sexual characters was not only related to the individual's ability to survive, but also to the individual's ability to find a mate. Indeed, sexual selection pressure promotes the development of strategies to display the best traits and to reflect the individual's quality. Darwin described two main mechanisms of selection: intra- and intersexual selection.

Intra-sexual selection describes the interaction between individuals of the same sex which compete for access to the other sex. This mechanism of selection affects the secondary traits and behaviours, such as body size and signalling characteristics, which are involved in competition for mating: better competitors can attract more mates and have more offspring. The intra-selection is more enhanced in species presenting an unbalanced operational sex ratio (OSR). It leads to stronger competition between individuals of the sex, where more subjects are willing to mate at a given time. In natural populations, an unbalanced sex ratio may occur because not all the females can be receptive at the same time, or males can have a refractory time between each pairing.

Inter-sexual selection describes the choice of one sex to mate with partners of the opposite sex and promotes the development of characters or ornaments (secondary sexual traits) that increase their attractiveness to individuals of the opposite sex (Darwin 1871). Since these characters should amplify attractiveness, the increased probability of mating opportunities should be the main outcome of this process. Following what Trivers (1972) assumed, females should generally be the choosier sex, because they invest more in terms of energy to each ovum or to offspring care (e.g. mammals), in comparison to the very small amount of energy dedicated by the male to each sperm. Therefore, males with extraordinary traits should be chosen by females (Darwin, 1871), to gain a high-quality mate and to increase both the survival rate and the attractiveness of their future offspring (Bateman, 1948).

However, a missing piece of Darwin's theory puzzle is the fact that many exaggerated traits are costly to produce and conspicuous, attracting not only mates but also predators and parasites. Many different theories have been developed to explain the origin and evolution of such exaggerated ornamental traits and mate preferences for such traits (Andersson, 1994; Clutton-Brock, 2009; Kokko & Jennions, 2008; Kokko *et al.,* 2003). For instance, the 'good genes' theory, assumes that females select males not only because of their attractiveness, but also because the males' displays and traits provide information about their viability-enhancing genes, which might increase the survival chances of the future offspring (Alcock, 2006; Møller & Alatalo, 1999). Within the broad list of models proposed to explain how individuals can recognize 'good genes' (Folstadt & Karter, 1992; Hamilton & Zuk, 1982; Zahavi, 1975), Zahavís 'handicap principle' (1975) is one of the best known. The grounding statement of the handicap principle is that exaggerated characters are honest signals which are costly to produce: males holding these elaborated traits present a handicap, which can decrease

their survival rate. This fact implies that only males in good condition can develop such traits and only individuals with good genes can survive (Zahavi, 1977). Females who choose these males will produce offspring which have a higher survival rate.

1.1.2 Sexual selection in acoustic communication

A remarkable feature of animal communication is the apparent honesty with which individuals advertise their quality. The term "costly signalling" often refers to Zahavi's (1975) idea, according to which signal honesty implies the payment of "handicaps" (strategic costs) by the signaller (Grafen, 1990). The strategic costs should maintain signal honesty, since it must be costlier for low-quality signallers to give a high-quality signal than it is for high-quality signallers (Grafen, 1990).

Several communicating species use acoustic signals for mate selection and pair formation (Searcy & Andersson, 1986; Catchpole & Slater, 1995; Gerhardt & Huber, 2002). The sender produces sounds to attract a conspecific receiver, who has to perceive the signal, and judge the signaller quality through the sexual cues emitted. In acoustic signalling insects, males produce sounds to attract conspecific females and to compete against rivals (Gerhardt & Huber, 2002; Robinson & Hall, 2002).

In Orthopteran species communicating acoustically, females often prefer male signals with high intensity, long duration and a high repetition rate (Gerhardt & Huber, 2002; Greenfield, 2002), that are energetically costly (Ryan & Keddy-Hector, 1992). Thus, based on how a potential mate uses intensity, frequency and/or the temporal structure in their signals (Schatral & Bailey, 1991), females can evaluate his quality. This precopulatory sexual selection of males, allows females to discriminate between available partners and to predict the benefits that the partner could offer (Gwynne 2001; Lehmann & Lehmann 2008). A high-quality signaller may honestly communicate a superior body condition, a characteristic that is likely to be relevant for female choice, via its acoustic performance (Schatral & Bailey, 1991; Wilgers & Hebets, 2015). Internal factors, such as body mass and size, can affect the acoustic trait produced and, in turn, the male's advertised quality (**Fig. 1-1**). Body size affects the intensity and frequency of the acoustic signal, and large males generally emit louder signals with a lower fundamental frequency (Bailey & Thiele, 1983; Latimer & Schatral, 1986). In crickets, signal duration and duty cycle are strongly positively correlated with body condition (Gray, 1997; Hack, 1997; Scheuber, Jacot, & Brinkhof, 2003; Shackleton, Jennions, & Hunt, 2005; Souroukis & Cade, 1993). Males in poor body condition can, therefore, only put a lower amount of effort into acoustic signalling (Holzer, Jacot, & Brinkhof, 2003; Ritchie, Sunter, & Hockham, 1998; Simmons et al., 1992; Wagner & Hoback, 1999), since they might incur higher costs of responding (Bretman, Gage, & Chapman, 2011). Acoustic production is an energetically demanding activity (Bailey, Withers, Endersby, & Gaull, 1993; Gerhardt & Huber, 2002) and it increases the individual's metabolic rate (Prestwich, 1994; Stevens & Josephson, 1977; Reinhold, 1999). The production of these conspicuous mate attraction signals might also lead to a further cost for signallers in terms of

predators - parasitoids attraction (Zuk & Kolluru, 1998). Clear examples are the predatory behaviours of bats towards crickets and katydids (Jones, Page, Hartbauer, & Siemers, 2011) and the parasitism of acoustically orienting flies on calling male crickets and katydids (Cade, 1975; Lehmann & Heller, 1998).

A high number of studies on bushcrickets (reviewed by Gwynne 2001) show that females generally prefer heavier males. Within the *Poecilimon* genus, the male body mass is an important factor for female choice (Lehmann & Lehmann 2008) and reflects the male's condition (Lehmann & Lehmann 2009). In *P. zimmeri*, Lehmann & Lehmann (2008) demonstrated that the signal is a crucial sexual cue, which allows females to discriminate between individuals of different body masses: without the males' signal input, the female's choice was performed randomly, independently of the male's mass. During mating, males invest in a large spermatophore including a spermatophylax that consists of food for the female, and the ampulla that holds the sperm (Wedell, 1994; Vahed & Gilbert, 1996; Gwynne, 2001). Previous studies showed that heavier and lager males produce a larger spermatophore (Wedell, 1993; Heller & Reinhold, 1994; Wedell & Sandberg, 1995; Gao & Kang, 2006) and are also better providers (Gwynne 1982; Lehmann & Lehmann 2008). The spermatophylax confers considerable and immediate benefits to the female, which receives nitrogen (Voigt et al. 2006) and carbohydrates (Voigt et al. 2008) through consuming it (Gwynne, 1982; Lehmann & Lehmann, 2008).

1.1.3 Morphology of production organs for sexually selected acoustic traits

Size is considered of major importance for a variety of biological questions (Schmidt-Nielsen, 1984; Brown & West, 2000; Houle *et al.,* 2011), and many characters under sexual selection show a positive allometric scaling with body size (Kodric-Brown, Sibly & Brown, 2006; Voje, 2016). A trait that confers a direct benefit in male competition and female choice will be favoured by a stronger directional selection resulting in an increase of the trait's size over the size (Bonduriansky & Day, 2003; Bonduriansky, 2007; Eberhard, Rodriguez & Polihronakis, 2009). Females of acoustically perceiving species select potential mates mainly through their acoustic signal (Gerhardt & Huber 2002). This requires mechanisms of male sound production, which ensure a reliable encoding of information about the male's quality in the signal (Gerhardt & Huber 2002). The sound-producing organs, like the traits of acoustic signals, evolve under sexual selection (Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005).

The structure of acoustic signals might change depending on internal factors, such as male body mass and size, and the physical characteristics of sound producing organs (Bennet-Clark, 1998). Developing and maintaining such selected structures, as well as all the traits involved in their function, may require high costs (Del Castillo & Gwynne, 2007). Individuals possessing a better body condition might have more resources to bear the costs of exaggeratedly expressed traits, which is supported by the scaling of song characters with body size or body mass, as found in other acoustic communicating

insects species (Rodríguez *et al.,* 2015). Larger individuals may benefit more from amplifying signals of highly competitive ability (Eberhard, Rodríguez, & Huber, 2018; Green, 1992; Petrie, 1992). Furthermore, high-quality individuals may be able to express or carry proportionally larger sexual structures (Andersson, & Simmons, 2006; Grafen, 1990; Zahavi, 1975).

In Orthoptera, the sound-producing apparatus is on the animal's wings, which are rubbed against each other to produce the sound. A modified toothed vein (stridulatory file) on the underside of the left wing is moved against the strong edge of the right wing (scraper) (Robinson and Hall, 2002) (**Fig. 2-1**). Sound impulses are generated only during the closing phase of wings with a "stridulatory movement", such as when the scraper on one wing hits the file teeth on the other. In stridulating bushcrickets, body size, signalling parameters and physiological processes are linked. In particular, the morphology of some elements of the sound-generating apparatus strongly affects the signal features (Morris & Pipher, 1972; Walker & Carlysle, 1975; Ritchie, Couzin, & Snedden, 1995; Orci *et al.,* 2005). Males with large-dimension sound-generating organs are able to produce low-frequency sound, whereas males presenting smaller organs emit songs with higher frequencies (Bennet-Clark, 1998; Podos & Patek, 2015).

1.1.4 Inter-individual variation of sexually selected acoustic traits and signal plasticity

Acoustic signals, like other sexually selected traits, are subject to other selection pressures (Andersson, 1994; West-Eberhard, 1983, 2014). As a result, trade-offs are expected in the allocation of resources to different vital functions (Del Costillo & Gwynne, 2007). According to theoretical models, natural selection will favour phenotypic responses that balance cost avoidance and resource acquisition (Ernande & Dieckmann, 2004). The ability to show plastic responses, which allows an organism to adjust its behaviour to changing environments and to increase its fitness (Snell-Rood, 2013; Sullivan-Beckers & Hebets, 2014), should be favoured by selection (Price, 2006; Robinson & Dukas, 1999). However, costs and limitations to plastic behaviour exist (DeWitt, Sih, & Wilson, 1998; Pigliucci, 2005; Van Tienderen, 1991), and their effect appears to vary substantially between individuals within a population (Briffa, Rundle, & Fryer, 2008; Dingemanse & Wolf, 2013). Some animals respond more efficiently to changes in the environment than others (Réale & Dingemanse, 2010), since not all organisms exhibit the "best" plasticity in all their traits (DeWitt, Sih, & Wilson, 1998; V*ia et a*l., 1995).

Due to the interplay existing between energetic constraints and the social environment, individuals of different body mass are likely to vary in their capacity to adjust their condition-dependent signal traits (Bretman, Gage, & Chapman, 2011). In **Figure 1-1**, factors causing the inter-individual variation in males' acoustic plastic response are represented schematically. They may be internal, such as body mass, body size and energetic condition of the signalling male (Bailey & Thiele, 1983; Forrest, 1983; Latimer & Schatral, 1986), or external, such as the number, quality and proximity of competitors in the environment (Gerhardt & Huber, 2002; Parrish & Hamner, 1997). Extrinsic

factors linked to the surrounding communication environment itself (i.e., density and number of signalling rivals, population mobility and operational sex ratio) can influence the magnitude of phenotypic plasticity (Bertram, Harrison, Thomson, & Fitzsimmons, 2013; Bretman, Gage, & Chapman, 2011) and even the individual's signalling tactics (Humfeld, 2013). Rapid fluctuations in the social context might favour behavioural plasticity: males must balance the costs of producing effective signals with the need to maintain their status in changing levels of signalling competition (Reichert & Gerhardt, 2012, 2013). The trade-off between being an efficient signaller and coping with the costs of expensive acoustic features (Mowles, Cotton, & Briffa, 2011; Reichert & Gerhardt, 2012; Smith & Harper, 2003) increases with the strength of the competition (Johnstone, 1997; Wilgers & Hebets, 2015). Empirical studies in many species show that animals often allocate more resources for signalling under highly competitive conditions (Gavassa, Silva, Gonzalez, & Stoddard, 2012; Griffith & Sheldon 2001; Reading & Backwell 2007; Wong & Svensson 2009). The socially mediated plasticity allows males to deal with high levels of acoustic competition, keep up their signalling efforts, and increase investment when it is expected to maximize mating success (Greenfield, 1994; Höbel, 2015; Klump & Gerhardt, 1992; Kuczynski, Gering, & Getty, 2016; Reichert, 2011).

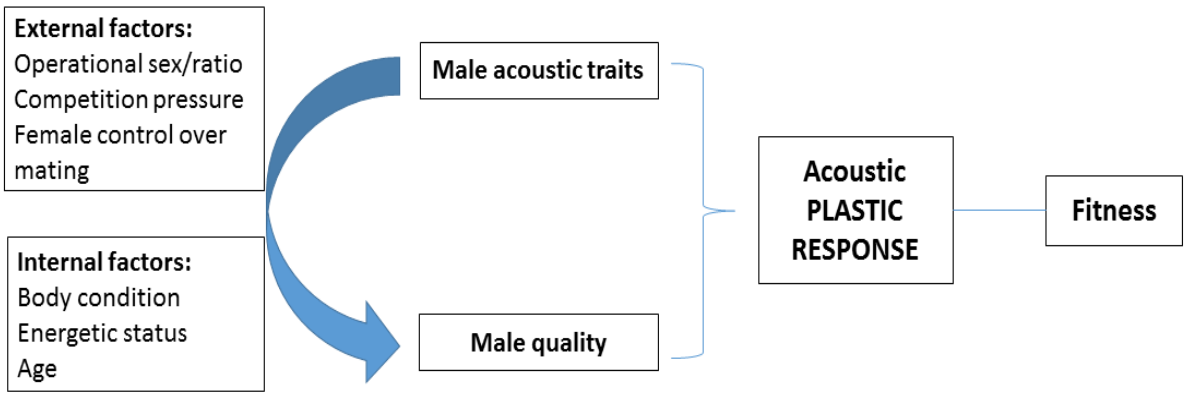

Figure 1-1. Schematic diagram of the factors affecting male acoustic plasticity and fitness benefits. Examples of external factors can be the operational sex/ratio which determines the likelihood that males will encounter intra-sexual competition before or after mating, the competition pressure, in terms of number, proximity and quality of rivals present in the surrounding environment and female control that will determine the strength of selection on male plasticity. Internal factors can be identified in the body condition, such as body mass and size, energetic status and age of the signalling males. The male plastic response is ultimately expressed in the acoustic traits that in turn will reflect the male's quality. The type of male plasticity that is ultimately selected for an expressed trait will lead to fitness returns in terms of increased reproductive success (number or quality of progeny produced).

Several studies have highlighted how males adjust their signals depending on variation in the surrounding communication environment. In Orthoptera, males can vary certain call features in

response to the vocal activity, the density and the proximity of signalling rival males (Hedrick, 1986; Latimer, 1981; Römer & Bailey, 1986).

In many bushcricket species, acoustic communication occurs in social settings composed of multiple signallers and receivers (McGregor & Peake, 2000), which are called choruses (Greenfield, 1994). These highly competitive social environments pose distinct challenges to effective communication, since males must ensure their signals stand out from those of their rivals (Bretman, Westmancoat, Gage, & Chapman, 2013; Höbel, 2015; Reichert & Gerhardt, 2012). In these aggregations, males engage in signal competition with their neighbours and may vary their call characteristics as a consequence of the vocal activity of the competitors (reviewed by Greenfield, 2005). A male signaller hearing the calls of conspecific rivals can gain acoustic information about their size, vigour, fighting ability or ability to attract females (Busnel, 1967; Greenfield & Shaw, 1983; Latimer & Schatral, 1986; Latimer, Romer & Bailey, 1986). Male bushcrickets can modify their sound interval and sound duration when rivals are signalling (Brush, Gian, & Greenfield, 1985; Dadour, 1989; Jones, 1974; Samways, 1976; Shaw, 1975). Given current debates in the literature regarding signalling strategies in both agonistic and mate-choice contexts (Arnott & Elwood 2009; Briffa & Elwood 2009; Botero, Pen, Komdeur, & Weissing, 2010), investigations of signal variability in competitive contexts are an important starting point for understanding the relationship between variation in resource allocation and signalling strategies.

1.1.5 The model organism: *Poecilimon*

Orthopteran insects (grasshoppers, crickets and tettigoniids) have served as optimum model organisms for studying both acoustic signalling and mating behaviour (Bailey, 1991; Gerhardt, Huber, 2002; Robinson, Hall, 2002; Greenfield, 2002). Especially in bushcrickets (Orthoptera: Tettigoniidae), there are two relevant aspects which determine their importance as study organisms:

1) the large nuptial gifts (spermatophore), which males produce and transfer as a food gift (spermatophylax) to a female during mating (ampulla) (Gwynne, 1997, 2001);

2) and the acoustic signals, which males produce for mate attraction and intra-sexual competition (Gwynne, 2001; Bailey, 1985; Greenfield, 2002).

This dissertation focuses on the ensiferan genus *Poecilimon*, (Fischer, 1853). Within this genus, there are 128 currently recognized species and subspecies (Otte, Eades & Nasrecki, 2005), which are quite uniform in terms of behaviour and life-history patterns (Heller & von Helversen, 1993). All the species present an obligate diapause and a univoltine lifecycle (McCartn*ey et a*l., 2008). Individuals use the wings to produce acoustic signals instead of flying and, depending on the species, two types of communication systems exist:

i. bidirectional, in which both males and females emit sound (Heller, 1990);

ii. unidirectional, in which only males produce sound signals and mute females, nearly wingless, locate them by a behaviour of positive phonotaxis (Heller, 1990; Heller & von Helversen, 1993).

In the thesis, I used two medium-sized, herbivorous species belonging to *Poecilimon* genus: *P. ampliatus* (Brunner Von Wattenwyl, 1878) (**Fig. S-2 right**) and *P. v. veluchianus* (Ramme, 1933) (**Fig. S-2 left**). In both species, males and females are flightless and only males signal (unidirectional communication system), with the "stridulatory movement" previously described (Robinson & Hall, 2002). Pairing takes place in a stereotypical sequence of behaviours, similar to what has already been described in *P. zimmeri* by Lehmann & Lehmann (2008) and during mating, a spermatophore is transferred by the male to the female. In *P. ampliatus*, the male acoustic production consists of stereotyped subunits, called "verses" (**Fig. 3-1**), which are separated by silent "pauses" (Heller & von Helversen, 1993; Heller & Reinhold, 1993). The dominant frequency of the *P. ampliatus* male songs is between 20 and 40 kHz (Heller & Lehmann, 2004; Heller, 1988). In *P. v. veluchianus*, males signal almost exclusively during the night (Heller & Reinhold, 1994), producing a pattern of sound unit called a "syllable" (Fig. 4-1), which is generated by a single down stroke movement of the forewings (Heller & Reinhold, 1994; Heller & von Helversen, 1993; Heller, 1998). These single syllables do not exceed one second in duration, contain less than twenty pulses and are separated by intervals of about one to three seconds (Heller 1990, 1998; Lehmann 1998). Henceforth, a further definition of the *P. v. veluchianus* males' signal will be used. The term "verse" will describe longer and repeated stereotyped subunits of sound, made by trains of a variable number of syllables separated by pauses of several seconds in between (**Fig. 4-1**).

1.2. Thesis structure

This thesis presents the results of a cumulative dissertation. Therefore, starting from a general introduction (**Chapter 1**), the thesis includes several parts that have already been published as peer-reviewed research articles (**Chapter 2**, **Chapter 3**) or are currently under revision for publication as a peer-reviewed article (**Chapter 4**). Due to the journal publication guidelines on the structure of the scientific work, redundant information occurs (e.g. in the introduction and methods parts). Finally, **Chapter 5** summarises the main findings of this thesis, states the main conclusions and formulates a potential outlook for following up the presented scientific results. Each chapter approaches different research questions and contributes to the overall production knowledge of the present thesis.

1.2.1 Research goals and questions

The overarching goal of this thesis is to integrate empirical findings from morphological, behavioural and acoustical studies to provide novel insights into the role that sexual selection plays in the morphology of sound generating structures and in the signalling plasticity of males' acoustic

production. In particular, the aims are firstly to understand the role that male body mass plays in the evolution of morphologies implied in signal production, and secondly, when combined with the external social environment, in the adjustment of males' signalling activity during competition for mates.

The following three research questions define the framework of this thesis:

Research Question I: Is the morphology of the signalling apparatus an honest cue of male quality, and how are these characteristics selected?

In bushcrickets, heavy males present various size benefits in regard to nuptial gift size (Lehmann & Lehmann, 2009) and female preferences (Lehmann & Lehmann, 2008). This leads to the hypothesis that the sound-producing structures are traits under sexual selection and that their size can be considered as a reliable predictor of size, mass and hence of male body quality. This research question is the core of **Chapter 2** and links the morphology of sound-generating organs with male body condition. Morphological data were obtained measuring the left-wing area, the total length of the stridulatory file, and the number, length and density of the teeth present o the middle part of the stridulatory file in males of *P. ampliatus* (**Fig. 2-1**; **Table S2-1**). As reference values of body mass and body size, respectively, the body weight and the length of the hind femur were measured.

The main objectives are:

i. to analyse the direction and the steepness of the regression slopes of the sound-producing structures with body mass and size;
ii. and to test which sound-producing organs show hyperallometric scaling with male body mass and size.

Research Question II: Is the acoustic production an honest predictor of male body condition when males compete against a rival placed at different distances?

In many bushcrickets, heavier males are more attractive to females. Heavier males present honest signal traits which transmit their body quality. However, in choruses, the male signalling activity can be further modified, since males act as both signaller and receiver. Their sound production can be adjusted depending on that of the neighbouring rivals. The sexually selected acoustic traits determining differences in males' quality are not yet clearly identified, and neither is the role played by the presence and proximity of a conspecific rival on the acoustic trait production. The study presented in **Chapter 3** aims to fill these gaps. Competing pairs of heavy and light males of *P. ampliatus* were tested under different competition levels, created by adopting different proximities for the rival (**Fig. S1-2**): placing the rival male either at the closest (inter-male distance: 10cm) or at the furthest distance (inter-male distance: 240cm), the highest and the weakest competition levels, respectively, were simulated. The main hypothesis is that heavy males always signal at higher

performance than the light rivals, even though both heavy and light males show a plastic response to the variation in the social context, increasing their acoustic production as the rivals get closer.

The main objects are:

i. to identify whether there is a signalling discrepancy between the two body mass classes of males tested both in isolation and under different competition levels;
ii. and to test whether, when contesting the rival at the closest position, both heavy and light males increase their acoustic production and show signal plasticity.

Research Question III: Is signalling plasticity a good predictor of body condition when males compete against rivals of different body mass and number?

In **Chapter 4** the study on male signalling behaviour under competition was extended, adopting P. v. veluchianus. In a semi-natural chorus as an experimental setup, the acoustic response of competing males was recorded (**Fig. S1-3 top**). Different competitive social environments were tested, combining both the number singing and the body mass of the rival signalling in the arena (**Fig. S1-3 bottom**): one light rival represents the minimal competition strength, while two heavy rivals identify the maximal competition strength. The aim of this last study is to investigate the effects of male body condition and different chorus composition on the plasticity of acoustic traits.

The main objectives are:

i. to test whether the focal males are able to alter the magnitude of their acoustic response depending on both the rival body mass and number;
ii. and to test whether both heavy and light males show signal plasticity, by comparing their signal production at the lowest and the highest acoustic competition pressure.

1.3. Supplementary Information

Figure S 1-1. *Poecilimon* study species in comparison. On the left side: male of P. ampliatus (photo: Gerlind Lehmann) and on the right side: male of P. v. veluchianus (photo: Martin Stoffel).

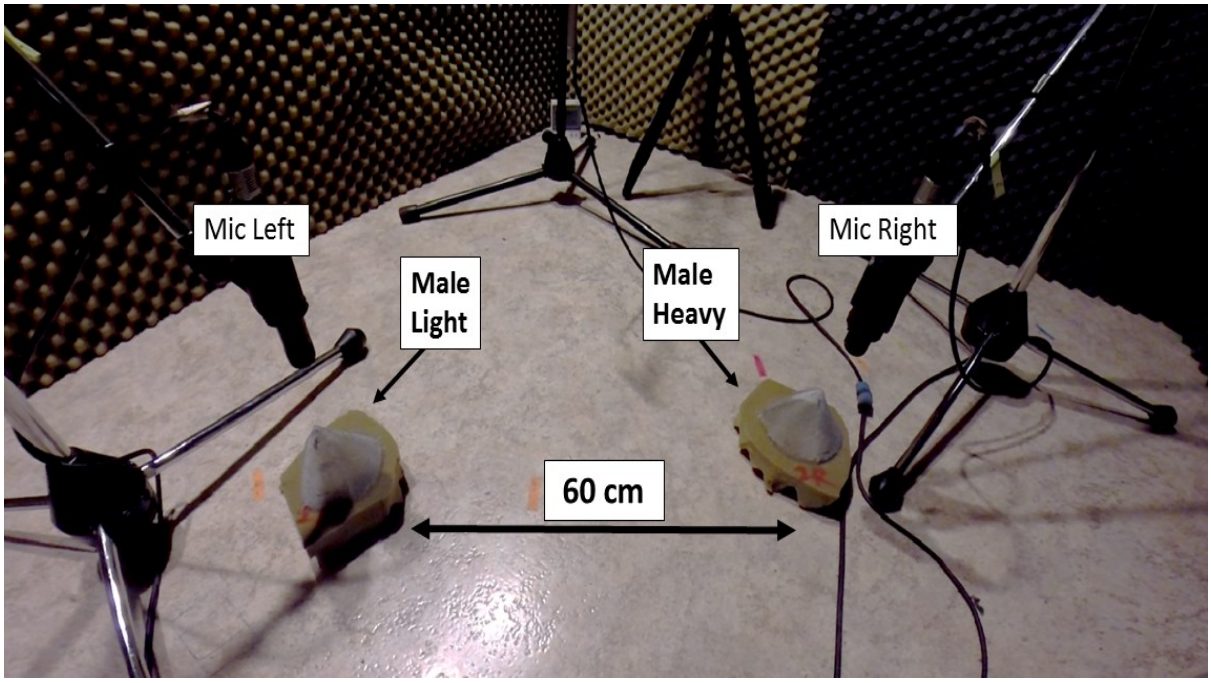

Figure S 1-2. Acoustic chamber experimental set up. Two microphones (Sennheiser MKH 40-P48), were placed on top each male (male light= left channel, male heavy = right channel). The double arrow line shows one competition treatment, adopting a distance of 60 cm between rival males.

Figure S 1-3. Field experimental arena set up. On top: real time acoustic recording of signalling response of the focal male, using UltraSound Gate 116-200 with 4 m cable and laptop. Bottom: within the enclosure (2 x 2 x 1 m), a single ultrasound microphone was placed on top of the focal male, which was at the vertex of an equilateral triangle. All the males (focal and rivals) were spaced equidistantly by 20 cm.

Chapter 2. Allometry of male sound-producing structures indicates sexual selection on wing size and stridulatory teeth density in a bushcricket

2.1. Abstract

The majority of bushcricket species (Insecta: Orthoptera: Tettigoniidae) use acoustic signals for mate attraction. Song production and the sound-producing structures, therefore, can be argued to be under sexual selection. The sound-producing structures might determine differences in the acoustic parameters of male songs, like the fundamental frequency. We investigated the morphology and scaling relationships of the sound production organs in males of the bushcricket *Poecilimon ampliatus*. Selection acting on morphological traits can be inferred from allometric relationships. Our results show that heavier and larger males have bigger sound-producing organs: wing area scales positively with body mass as well as with body size. The length of the stridulatory file and the stridulatory teeth length both increase with wing area. In contrast, the number and density of teeth on the stridulatory file decrease in heavier males. As a result, males with larger wings and longer stridulatory files have a reduced teeth density. Positive isometric scaling was detected for the length of stridulatory teeth, as well as negative isometry for teeth number and teeth density. Wing area in turn seems to be under positive sexual selection, as the relationship of wing size on male body size show a steep positive slope (β = 2.4). The morphological results indicate positive selection on sound-producing structures and are in line with the female preference for heavier males in mate choice experiments.

2.2. Introduction

In species using acoustic communication for mate attraction, the acoustic signals and the sound-producing structures evolve under sexual selection (Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005). Developing and maintaining such selected structures, as well as all the traits involved in their function require high costs, which might depend on male condition (Del Castillo & Gwynne, 2007). Songs can be considered as honest signals (Grafen, 1990) and their acoustic structure might change depending on male quality and physical characteristics of sound generator organs (Bennet-Clark, 1998). Males with poorer body condition produce less efficient signals, compared to males of higher body condition (Podos & Patek, 2015).

In Orthoptera, sound is produced by stridulation, rubbing two body parts against each other. Male bushcrickets (Orthoptera: Tettigoniidae) stridulate by using a modified cuticular toothed vein, the stridulatory file, located on the underside of the left wing, which is moved against a scraper, the strong edge of the right wing. Sound impulses are generated when the scraper on one wing hits the file teeth on the other. The number of teeth present on the stridulatory files is fixed at moulting (Gerhardt & Huber, 2002).

In stridulating bushcrickets, body size, calling parameters and physiological processes are linked, in particular, the morphology of some elements of the sound-generating apparatus strongly affects the song (Morris & Pipher, 1972; Walker & Carlysle, 1975; Ritchie, Couzin & Snedden, 1995; Orci *et al.,* 2005). Moreover, the file-tooth arrangement may affect the sound component such as the amplitude (Montealegre-Z & Mason, 2005). The fundamental frequency is one of the most important features of the song and is mainly defined by speed of the wing movement and the spacing of teeth along the stridulatory file (Podos & Patek, 2015). Males with large-dimension sound-generating organs are able to produce low-frequency sound, whereas males presenting smaller organs are emitting songs with higher frequencies (Bennet-Clark, 1998; Podos & Patek, 2015).

The brachypterous genus *Poecilimon* exhibits high species richness (Heller, 1984; Willemse & Heller, 1992). Whereas the majority of *Poecilimon* species use acoustic duetting for mate attraction and finding, our study species *Poecilimon ampliatus* belongs to the species with secondary unidirectional sound communication. The reversal to unidirectional communication system evolved within the genus *Poecilimon* (Strauß, Lehmann & Lehmann, 2014), with males acoustical signalling, while females are nearly wingless, mute and locate males by phonotaxis (Heller, 1992; Heller & von Helversen, 1993). Body mass selection by female choice seems to be severe in unidirectional signalling *Poecilimon* species, as heavier males produce songs that are more attractive to females (Lehmann & Lehmann, 2008) and provide larger nuptial gifts (Lehmann & Lehmann, 2009). The female choice of heavier males is adaptive, as female bushcrickets gain energetic benefits by consuming the enlarged spermatophylax (Voigt *et al*., 2006, 2008; Lehmann & Lehmann, 2016).

Size is considered of major importance for a variety of biological questions (Schmidt-Nielsen, 1984; Brown & West, 2000; Houle *et al.,* 2011), and many characters under sexual selection show positive scaling with body size (Kodric-Brown, Sibly & Brown, 2006; Voje, 2016). Sexual selection is generally thought to favour the evolution of positive allometric traits, as larger trait size confers a direct benefit during sexual competition and female choice, resulting in stronger directional selection on trait size than on body size (Bonduriansky & Day, 2003; Bonduriansky, 2007; Eberhard, Rodriguez & Polihronakis, 2009). Better conditioned individuals have the resources to bear the costs of exaggeratedly expressed traits, which is supported for the scaling of song characters with body size or body mass in a recent overview of acoustic communication (Rodríguez *et al.,* 2015). However, multiple costs of sexually selected characters reduce evolvability, leading to conserved relationships over time (Hansen & Houle, 2004; Frankino *et al.,* 2005, 2007; Pélabon *et al.,* 2014; Voje *et al.,* 2014; Bolstad *et al.,* 2015). The concept of allometry is used for scaling relationships, allowing for comparison between studies and traits (Warton *et al.,* 2006; Klingenberg, 2016; Mirth, Frankino & Shingleton, 2016; Stillwell *et al.,* 2016). Scaling traits of individuals of the same developmental stage, in our case adult, are called static allometry. Developmental processes may create slopes of different steepness between a trait and body size or mass. The allometric equation of log–log transformed measurements allows easy comparison of slopes; when trait size scales linearly with body size, the slope has a value of |1|, the relationship is called isometric. This isometry can be positive (slope = 1) or negative (slope = -1), see Fig. 2-2. Scaling of traits disproportional with body size is classified as hyperallometry (slope > 1 for positive hyperallometry and < -1 for negative hyperallometry) or hypoallometry (slope between 0 and 1 for positive hypoallometry and between 0 and -1 for negative hypoallometry) (Bonduriansky & Day, 2003; Mirth *et al.,* 2016; Stillwell *et al.,* 2016). The usage of positive and negative scaling relationships differs from the wording of negative allometries (slope < 1), which equals a positive hypoallometry in our definition and positive allometries (slope > 1), which means a positive hyperallometry in our sense.

Scaling of bushcricket signal parameters was comparatively examined by Montealegre-Z (2009), reporting a positive correlation between body size and length of forewings, and stridulatory file, as well as a negative correlation between tooth density and stridulatory file length between species. The size of the stridulatory teeth might also be under selection, as they get lost or damaged with continuous use (Ritchie *et al*., 1995).

To link the morphology of sound-generating organs with male body size and body mass, we studied adult males of *Poecilimon ampliatus*. Under the assumption that sound-producing structures are under sexual selection, we expect hyperallometric relationships with slopes greater than 1. In line with the size benefits of heavier males in respect of nuptial gift size (Lehmann & Lehmann, 2009) and female preferences (Lehmann & Lehmann, 2008), we expect that morphological traits of the sound-production organs show hyperallometric scaling with male body mass and size.

2.3. Methods

Around 200 males of *Poecilimon ampliatus* (BRUNNER VON WATTENWYL, 1878) were collected in the field as nymphs in May 2015, near the village of Gabrče (45°71'29.9" N, 14°02'28.8" E), Slovenia. All individuals were individually caged and transferred to the laboratory, at the Institute of Biology at the Humboldt University Berlin. The animals were fed with *Taraxacum* leaves and pollen ad libitum, kept at a stable temperature between 23 and 25°C and a day–night rhythm of 15:9 h. A number of 55 males were preserved in 70% alcohol. On average, they were 23 days old (range 20–27 days).

2.3.1 Morphological measurements

Before storing the samples in alcohol, male body mass was measured (KERN ALT 100-5 AM; accuracy ±0.01 mg). As hind femur length can reliably be used as an indicator of overall body size, the left hind femur and the left wing were removed from males' bodies. Morphological traits were measured with the help of a digital microscope (VHX-1000, Keyence Corporation, Neuilsenburg, zoom object: VH-Z00W) at the following magnifications (in parenthesis): hind femur length (20x), wing area (50x), length of the stridulatory file (100x), teeth number (150x), teeth density and teeth length (200x). The left wing area was evaluated, considering the area included within the polygon made retracing the wing edge with the cursor of the digital microscope. We measured the total length of the stridulatory file, which is located on the ventral side of the left wing (Fig. 2-1a).

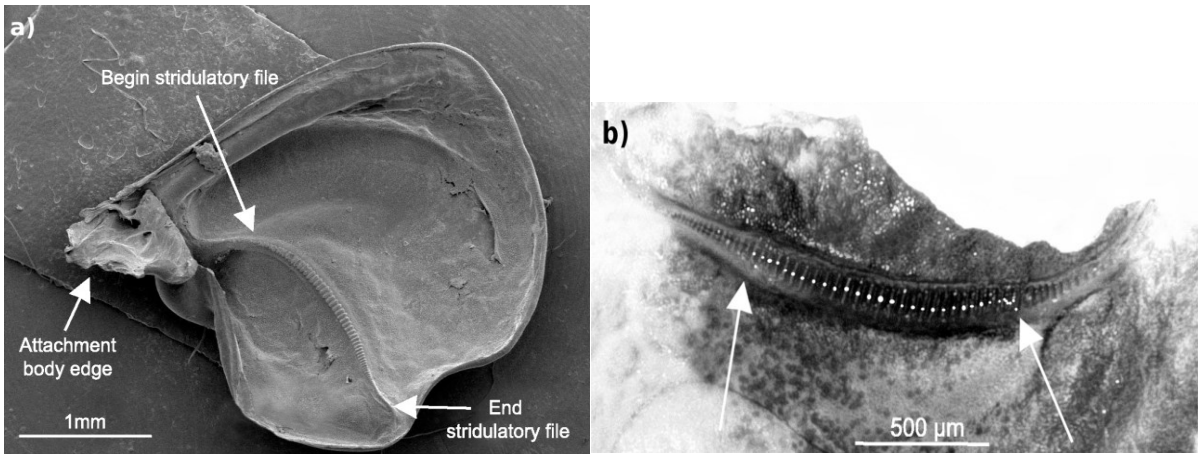

Figure 2-1. Pictures of the left forewing of a *P. ampliatus* male. (a) Ventral side of the wing showing the position of the stridulatory file, spanning from the base of the wing (to the left) all the way to the wing margin; (b) Detail of the stridulatory file. Arrows mark the borders of the defined middle file region where teeth were counted (white spots).

The stridulatory file is slightly curved and contains a densely packed row of stridulatory teeth. These teeth are chitinous and their size is gradually reduced towards the extremities. As the smaller teeth located at the outer and inner ends of the file were hardly discernible (Fig. 2-1b), only teeth in

the middle region part of the stridulatory file have been considered. Within this middle part, each tooth was counted. Dividing the total number of teeth by the length of the "middle file region", we calculated the teeth density. Additionally, the maximum length of teeth, transverse to the stridulatory file direction, was measured for this middle file region (Fig. 2-1b).

2.3.2 Allometry of sound-generating organs

In an allometric relationship, the observed variables are related by the equation: $Y = \gamma * X^{\beta}$. All variables were logarithmically transformed (Warton *et al.*, 2006) as this technique has the potential to normalize distribution of the data, thereby reducing the heteroscedasticity (Kerkhoff & Enquist, 2009; Packard, 2009; Packard, Birchard & Boardman, 2011). It also simplifies the comparisons of proportional relationships, allowing for visual inspection (Glazier, 2013; Klingenberg, 2016; Mirth *et al.,* 2016). After $\log_{10}$ transformation, our equation becomes linear: $\log y = \log \gamma + \beta \log x$. The scaling exponent ($\beta$) is equivalent to the slope of the fitted line (Falster, 2006). Each character of the sound-generating organ (wing area, stridulatory file length, number, density and length of teeth) along with body mass and hind femur length was tested against the null hypothesis of isometry, with a slope of 1 or -1. Allometric relations between body dimensions and wing parameters were analysed using standardized major axis (SMA) line fitting method because it assumes no direction of the relationship between traits and reflects the variances in both directions (Warton *et al.,* 2006; Smith, 2009; Stillwell *et al.,* 2016).

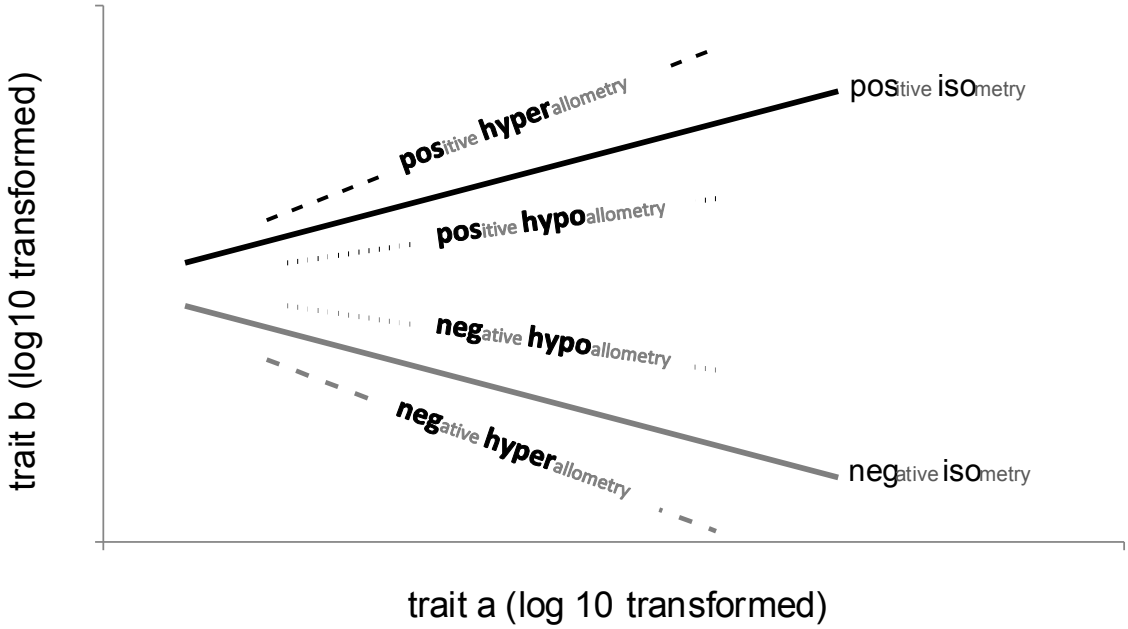

Figure 2-2. Schematic illustration of the different types of bivariate allometries. Trait b is regressed against trait a, both are transformed by the logarithm to the base of 10. Thick lines show an isometric change in trait size with a slope of 1.0, either the trait increases (positive isometry in black) or

decreases (negative isometry in grey). A change in relative trait size with a slope larger than 1 is called hyperallometry (black dashed line = positive hyperallometry, grey dashed line = negative hyperallometry). If trait b changes with a slope smaller than 1, we will call it hypoallometry, either positive (black dotted line) or negative (grey dotted line).

Signal traits (here, signal length) may vary in how they scale on individual body size: hyperallometry: steep or positive allometry ($b > 1$), isometry: proportional scaling ($b = 1$) and hypoallometry: shallow or negative allometry ($b < 1$) (Fig. 2-2). For calculating, we used the package SMATR (Falster, Warton & Wright, 2006; Warton & Ormerod, 2007; Warton *et al.,* 2012) in the R environment (R Core Team 2015).

2.3.3 Multiple-regression analysis of sound-generating organs

The $\log_{10}$-transformed measurements were used in multiple-regression analyses. We performed stepwise multiple-regression analyses with a backward elimination method. Starting to fit the first model having the wing area as dependent variable (Y), we tested which proportion of the variance of this variable is explained by the relative contribution of body mass, femur length or stridulatory file.

Equation: Wing area = $\alpha + \beta_1$ Body mass + β_2 Femur length + β_3 Stridulatory file length

Afterwards, one after the other stridulatory length, number, density and length of teeth were considered as dependent variables and a model for each of them was developed. Body mass, femur length, wing area and in consecutive steps stridulatory file length were included in the model as independent variables, using R version 3.2.2 “Fire Safety” (R Core Team, 2015).

2.3.4 Qualitatively analysis of teeth condition

A qualitative evaluation of the morphological condition of teeth of the stridulatory file was carried out. We performed this by analysing pictures from a SEM microscope (JSM-6060LV, JEOL Ltd.) after drying and sputtering the samples with a 30-nanometre gold layer (Fig. 2-1a, 5).

2.4. Results

Poecilimon ampliatus males are small compared with other *Poecilimon* species, with a body mass of 362.50 ± 49.18 mg (mean ± SD) and a hind femur length of 13.94 ± 0.53 mm (mean ± SD) (Table S 2-1).

Starting to consider the scaling relations between morphological traits, 14 of them turned out to change significantly in correlation with another trait (Table 2-1).

As body mass and body size are interconnected and both influence the majority of the sound-producing structures, we separate influence of either characters by using multiple-regression analyses (Table 2-2, Fig. 2-4).

The stepwise multiple regressions reveal that the wing area scales positively with body mass (Fig. 2-3a) as well as body size. The coefficient of determination for the entire model (R^2) is 0.37 and indicates a good fit to the model (Table 2-2). Regarding the single factors, body mass explains more of the variation in wing area (R^2 = 0.30) than body size (R^2 = 0.18). The slope of 0.65 ± 0.05 SE for this regression shows significant hypoallometric scaling of wing area with body mass (Table 2-1); this indicates selection on lighter males to invest into larger wings. In contrast, wing area increases hyperallometrically more than two times faster than femur length with a slope of 2.41 ± 0.04 SE. Therefore, larger males show increased wing size, suggesting positive selection.

Table 2-1. Significant relationships of morphological sound-generating structures using standardized major axis (SMA).

	F	d.f.	*P*	R^2	P_{SMA}	Slope	95% CI		SE
Body mass									
Femur length	4.81	51	**0.033**	0.09	**0.000**	0.27	0.21	0.35	0.04
Wing area	21.20	51	**0.000**	0.30	**0.000**	0.65	0.51	0.83	0.08
Stridulatory file length	12.02	51	**0.001**	0.19	**0.000**	0.40	0.31	0.51	0.05
Teeth number	6.59	51	**0.013**	0.11	0.328	-1.14	-1.49	-0.87	0.16
Teeth density	7.15	51	**0.010**	0.12	**0.000**	-0.42	-0.55	-0.33	0.10
Body size (femur length)									
Wing area	11.85	51	**0.001**	0.18	**0.000**	2.41	1.87	3.11	0.31
Stridulatory file length	5.26	51	**0.026**	0.09	**0.008**	1.45	1.10	1.91	0.20
Teeth density	4.82	51	**0.033**	0.09	**0.001**	-1.59	-2.09	-1.21	0.30
Wing area									
Stridulatory file length	12.48	51	**0.000**	0.19	**0.002**	0.62	0.46	0.84	0.08
Teeth density	33.52	51	**0.000**	0.40	**0.002**	-0.67	-0.85	-0.53	0.08
Teeth length	8.57	51	**0.050**	0.14	0.577	0.93	0.74	1.25	0.12
Stridulatory file length									
Teeth density	19.99	51	**0.000**	0.28	0.667	-1.06	-1.38	-0.81	0.14
Teeth length									
Teeth density	9.92	51	**0.003**	0.16	**0.027**	-0.74	-0.97	-0.56	0.10
Teeth density									
Teeth number	18.43	51	**0.000**	0.27	**0.000**	2.61	2.03	3.35	0.29

The equation adopted: log *Y* = intercept + slope*log *X*. P_{H0}: Isometric slope (β = 1); *F* = *F*-value, d.f. = Degree of Freedom, *P* = *P*-value correlation, R^2 = Coefficient of Determination, P_{SMA}= *P*-value for SMA, testing for a deviation of the slope from 1 or -1, CI = Confidence Interval of the slope at 95%, SE = Standard Error. *P*-values in bold highlight significant correlations.

Wing area is a main predictor of sound-producing structures. Considering the stridulatory file length as dependent variable, the model significantly includes only wing area as explanatory morphological parameter. In the single regression, 19 per cent of the variation in stridulatory file length is explained by the wing area (Table 2-1), and this value increases to 22 per cent after elimination of other non-significant effects (Table 2-2). The length of stridulatory file shows a positive hypoallometry close to 2/3 (slope 0.62 ± 0.08 SE) compared to the wing area (Fig. 2-3c).

Table 2-2. Stepwise multiple-regression values on morphological variables with backward elimination method to select the independent variables into the model.

	β	SE	t	$Pr(>\|t\|)$	F	d.f.	R^2	P
Wing area					11.2	49	0.37	0.000
Body mass	0.25	0.08	3.04	0.004				
Femur length	0.64	0.29	2.23	0.030				
Stridulatory file length					8.40	50	0.22	0.001
Wing area	0.19	0.09	2.01	0.049				
Teeth number					6.60	51	0.10	0.013
Body mass	-0.40	0.16	-2.57	0.013				
Teeth density					22.64	50	0.45	0.000
Stridulatory file length	-0.37	0.13	-2.74	0.008				
Wing area	-0.36	0.08	-4.29	0.000				
Teeth length					8.57	51	0.13	0.005
Wing area	0.36	0.12	2.93	0.005				

β = Slope, SE= Standard Error of the regression coefficient, t = t value, $Pr(>|t|)$ = P-value for the t statistics, F = F-value, d.f. = Degree of freedom, R^2 = Coefficient of Determination, P = P - value of the model.

Teeth length scales proportional with wing area, males with larger wings have equally larger teeth (isometry, Fig. 2-3e). Variation in teeth length is moderate and wing area explains 13 per cent of the variation (Table 2-2). The density of teeth is influenced by two factors, 45% of the model is described by the combination of wing area and stridulatory length (Table 2-2). In the multiple-regression analysis, the explanatory variables (wing area and stridulatory file length) present similar negative slopes (β = -0.36 and -0.37, Table 2-2).

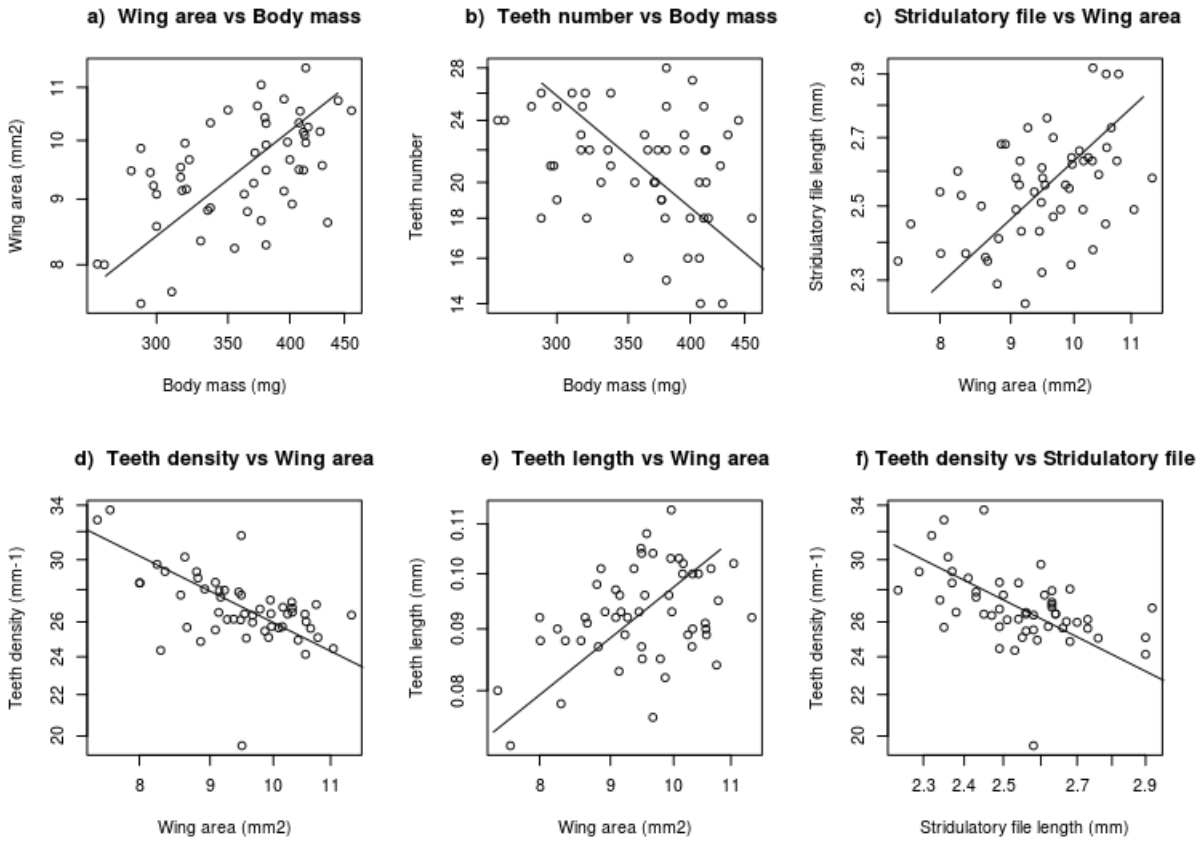

Figure 2-3. (a–f). Scaling of sound-producing traits, which were found significant after multiple-regression analysis, see Table 2. Details of these allometric relations, using standardized major axis (SMA), are presented in Table 1. Correlation between (a) Wing area and body mass, (b) Teeth number and body mass, (c) Stridulatory file length and wing area, (d) Teeth density and wing area, (e) Teeth length and wing area and (f) Stridulatory file length and teeth density.

In the single regression analysis, the direction of the relationships of both factors clearly differ (Table 2-1): whereas the decline in teeth density is almost equal to -1 when related to the stridulatory file length, presenting in this case a negative isometry (Fig. 2-3f, Table 2-1), we find a negative slope of -0.67 between teeth density and wing area (Table 2-1). It is interesting to note that the number of stridulatory teeth on the file is independent of the other sound-producing structures and is related only to body mass (Table 2-2). This relationship is proportional in the opposite direction, teeth number declines isometric when body mass increases (Fig. 2-3b).

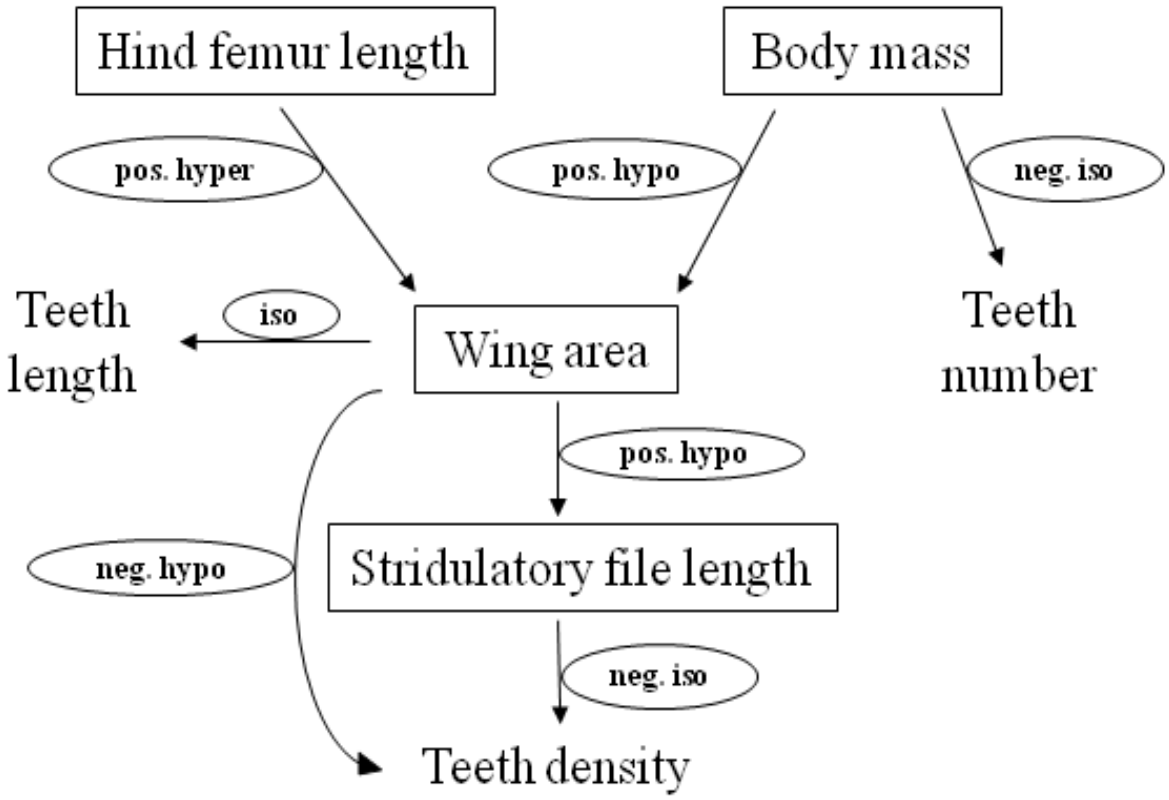

Figure 2-4. Summary of the main significant relationships, after allometric and multiple-regression analysis. neg. iso = negative isometry, the two character changes proportional, but inversely, in size – indicative of stabilizing selection; pos. hyper = positive hyperallometry, character sizes change disproportionally, indicative of positive selection; pos. hypo = positive hypoallometry, character sizes change disproportionally, indicative of negative selection; neg. hypo = negative hypoallometry, character sizes change disproportionally, but inversely, indicative of opposing stabilizing selection.

In summary, the seven significant relationships in the multiple- regression analysis (Table 2-2) provide a quite clear picture (Fig. 2-4): wing area increases with body mass and body size. Equally, teeth length and stridulatory file length increase as wing area increases. In contrast, teeth number and teeth density both decrease with body mass, wing area and stridulatory file length. The majority of factors changes either proportionally (= isometric) with the other factor or show hypoallometric scaling. This indicates a stabilizing selection or a selection pressure for lighter males to increase song structures relative to heavier males. Only wing area shows hyperallometric scaling, when regressed against body size measured as hind femur length. Those larger males bear not only absolutely but also disproportionally larger wings than smaller males (Fig. 2-4).

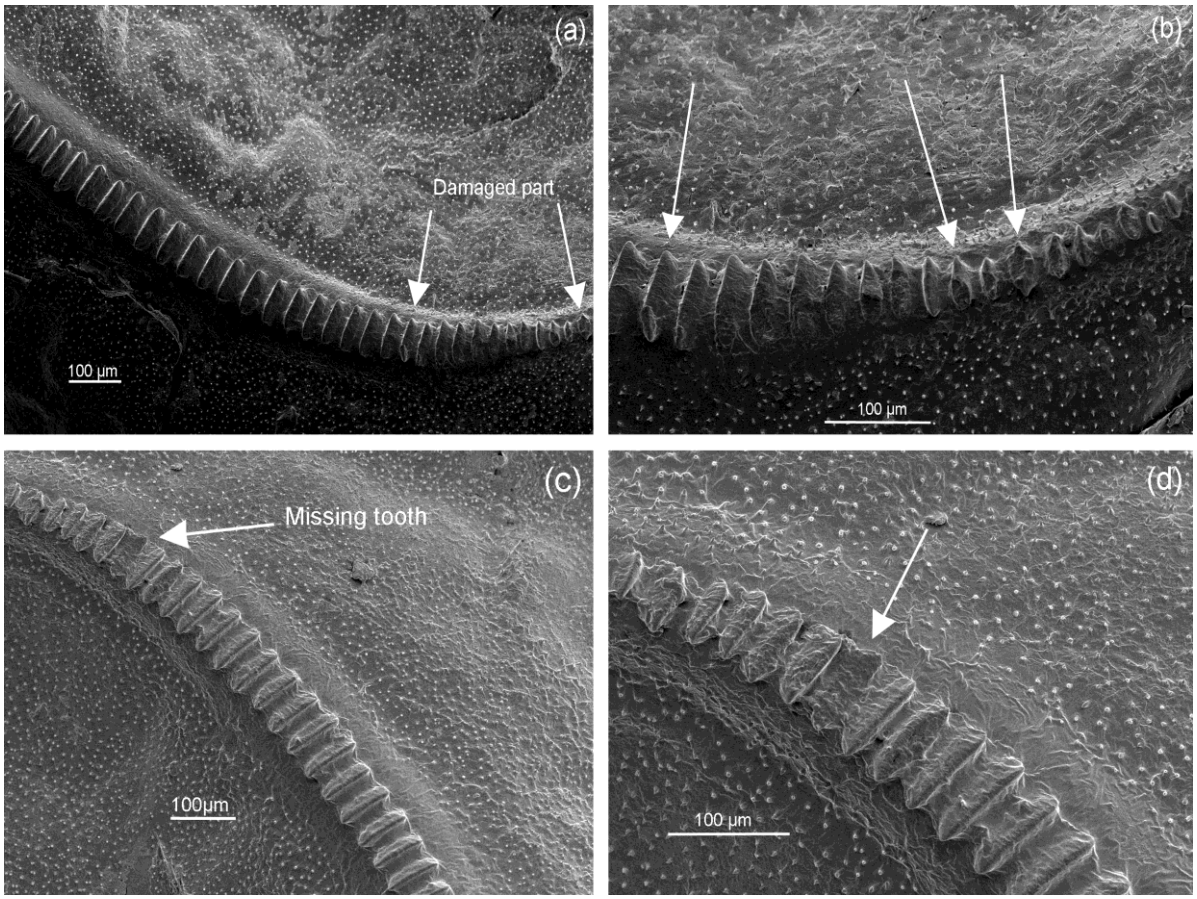

Figure 2-5. SEM microscopy pictures. (a) Stridulatory file with damaged teeth; (b) Enlargement; (c) Stridulatory file with missing tooth; (d) Enlargement.

Stridulatory teeth condition

Each tooth was rectangular shaped, and the larger ones were distributed in the medium region of the stridulatory file with a gradual reduction in size towards the extremities. In our sample of 55 males, nearly one-fifth (18%) have worn or missing teeth (Fig. 2-5). The smaller teeth were located at the outer and inner ends of the stridulatory file, showing an asymmetric morphology, irregular shape and different kind of damages. In some cases, the teeth had broken edges (Fig. 2-5a, b), whereas in others the tooth was completely missing (Fig. 2-5c, d).

2.5. Discussion

Sound-generating structures in our study species *P. ampliatus* scale with body size and body mass, however, the direction of the relationships and the steepness of the regression slopes vary considerably between traits. Larger and heavier males bear larger wings, with longer stridulatory files and greater stridulatory teeth lengths. Body mass plays a crucial role in determining the teeth number and density. However, stridulatory teeth show a size constraint, with increasing teeth length they need more space. Therefore, heavier males have larger but fewer teeth, which are further parted. Our results showed the

general trends for relationships of sound and body size found in a comparative study of 58 bushcricket species: in this cross-species comparison, the size of the wing, the sound-generating mirror, the stridulatory file and the stridulatory teeth all scale positively with male body size (Montealegre-Z, 2009). Despite this convergence in the results scaling slopes are different between species and within our species. Wing area in our study shows a hyperallometry when regressed on body size, which might be a hint for wing size to be under positive selection. Slopes greater than 1 were not found in the comparative study (Montealegre-Z, 2009) suggesting larger stabilizing selection in the macroevolutionary trend.

The proposed positive selection on wing size may correspond to the functional consequences of larger size for the acoustic parameters in a male's song. Larger sound-generating organs are generally associated with the production of lower-frequency signals (Bennet-Clark, 1998; Podos & Patek, 2015), a common pattern also found comparing species of bushcrickets (Del Castillo & Gwynne, 2007). This pattern needs still to be verified in our species, as a study in crickets detected increasing sound apparatus sizes with larger male body size but was unable to detect an association with song frequencies (Miyashita *et al*., 2016). However, cricket and bushcricket hearing systems differ fundamentally (Stumpner & von Helversen, 2001): crickets have matching frequency filters (Kostarakos, Hennig & Römer, 2009), whereas bushcrickets possess tonotopically organized ears tuned to broadband frequencies (Stumpner, 1996; Stumpner & Nowotny, 2014). Therefore, cricket hearing might select frequencies to be largely invariant; on the other hand, in bushcrickets frequency seems to be an honest signal for body size. Females discriminate for low-frequency song, which is associated with larger male body size (Latimer & Sippel, 1987; Gwynne & Bailey, 1988). *Poecilimon* females consistently prefer heavier males based on their song (Lehmann & Lehmann, 2008), with heavier males providing larger nuptial gifts at mating (Lehmann & Lehmann, 2009). Female choice of heavier males is adaptive, as females energetically benefit from consumption of the nutritious spermatophylax (Voigt *et al.,* 2006, 2008; Lehmann & Lehmann, 2016). The development of larger wings, a longer stridulatory file and larger stridulatory teeth, however, might be constrained by several factors like the costs of sexually selected features (Simmons *et al*., 1992; Podos & Patek, 2015), genetic covariance with other traits and the resources available during ontogenesis (Bonduriansky, 2007). Bushcricket males face high costs in producing the nuptial gift, which in *P. ampliatus* represents 20% of a male's body mass (Lehmann & Lehmann, unpublished data).

Depart from the positive allometry found for wing size; the length of the stridulatory teeth scales isometric with body size. For the stridulatory teeth we found a "usage effect", teeth get worn and broken over time. The abrasion occurred mainly on the smaller teeth at both ends of the stridulatory organ. The altered sound pulses produced by damaged teeth might be perceived by females and influence their mate choice (Ritchie *et al*., 1995). We therefore believe that teeth size is under selection, with larger teeth being better in withstanding abrasion and to function for longer time.

Interestingly, the length of the stridulatory file scales hypoallometrically with wing area, showing a shallow slope less than 1. Two alternative selection modes can explain a hypoallometry: there might be opposing or negative selection against further increase, especially in large males, or smaller males are under enhanced pressure to compensate their body size handicap by enlarging the file length (Eberhard, 2009). However, the selective forces and the direction and the magnitude of natural and sexual selection to produce the variety of scaling relationships in the sound structures in P. ampliatus remain unknown.

2.6. Conclusions

We could show that heavier males develop larger wings with longer stridulatory files. Heavier males also bear larger but fewer, less densely spaced stridulatory teeth than lighter individuals. Thus, sexual selection for larger nuptial gift size and honest signalling might be coupled to positive selection on the size of sound-producing structures in a bushcricket.

2.7. Acknowledgement

We gratefully acknowledge K. Rademann and his team, Humboldt University Berlin, Institute of Chemistry, for using the SEM Microscope and providing all the technical support. We also thank G. Scholtz, Institute of Biology, Humboldt University Berlin, for the use of the digital microscope. We are grateful to D. I. Warton, who has provided his feedback during the SMA analysis. We also thank G. Arpaia, L. Azzani, L. J. Eberhart-Phillips, E. Papale, M. Rizzuto and J. Strauß for their comments on an earlier version of the manuscript. A special thanks to A. Bonaduce, A. Lehmann and to my Ph.D. colleague N. Wulff for the useful discussions. Marianna Anichini was financially supported by the Elsa Neumann-Stipendium des Landes Berlin Grant from the Humboldt University of Berlin and by the German Society of General and Applied Entomology (DGaaE).

2.8. Supplementary Information

Table S 2-1. Morphological measurements of body parameters and song generator structures.

	Minimum	1st Qu.	SD	Mean	3rd Qu.	Maximum
Body mass / mg	264.00	319.20	49.84	362.50	406.50	457.00
Femur length / mm	12.69	13.53	0.54	13.94	14.45	15.00
Wing area / mm^2	7.46	8.92	0.89	9.51	10.16	11.39
Stridulatory file length / mm	2.24	2.44	0.15	2.54	2.63	2.91
Teeth number	14.00	18.00	3.41	21.04	23.75	28.00
Teeth density per mm	0.024	0.026	2.00 e-3	0.027	0.028	0.034
Length teeth / mm	0.07	0.09	8.37 e-3	0.093	0.10	0.11

Minimum = Minimum value, 1st Qu. = First quartile, SD = Standard Deviation, Mean = Average value, 3rd Qu. = Third quartile, Maximum = Maximum value.

Chapter 3. To compete or not compete: bushcricket song plasticity reveals male body condition and rival distance

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3.1. Abstract

Males of several communicating animals, including insects, use acoustic signals to attract a sexually receptive conspecific partner. In the orthopteran chorusing genus *Poecilimon* (Tettigoniidae), male signalling as well as female preference can be related to male body condition and to the social environment. Song is thought to be an honest signal of male quality, and song characteristics are therefore often important for sexual and social selection. At the same time, signal expression is plastic, and this plasticity depends on the quality of the individual signaller, the acoustic components preferred by females and rivals' body condition and proximity. Using the bushcricket species *Poecilimon ampliatus* as a model, we investigated how both internal (body condition) and external (level of competition) factors affected the expression of temporal song characteristics. We show that both factors significantly affect acoustic signalling activity-when competing against light rivals, heavy males adjusted the characteristics of their songs to different social conditions. However light males competing against a heavy rival showed less plasticity in their acoustic signals across social conditions. During the most escalated competition, heavier males increased their acoustic signal investment up to the maximum level, signalling with longer verses and higher duty cycles, in comparison to all other treatments. Body individual condition and the social environment affected male acoustic signal activity, which suggests that these factors mediate the allocation of resources for signalling and different strategies adopted in competition. The adaptive plasticity of acoustic signals in this species raises new questions about the potential role that this process could play in natural choruses, where more than two competitors are signalling simultaneously.

3.2. Introduction

Sexually selected traits are also subject to other natural and social selection pressures present in the environment (Andersson, 1994; West-Eberhard, 1983, 2014). Consequently, individuals which invest in morphologies and behaviours that provide an advantage in fitness (Andersson, 1994; Byers, Hebets, & Podos, 2010) may adjust these traits in response to factors internal to the individual such as age and energetic state, or external such as predation risk and population density (Kasumovic, 2013). Thus, this phenotypic plasticity can have an important impact on the outcome of sexual selection (Snell-Rood, 2013; Zuk, Bastiaans, Langkilde, & Swanger, 2014). For instance, in competition for mates, a male may adjust his mate-attraction signals according to his perception of the density and types of surrounding rivals (Bretman, Gage, & Chapman, 2011).

In many species of frogs and insects, the male acoustic communication signals evolved under sexual selection (Gerhardt & Huber, 2002; Greenfield, 2002; Robinson & Hall, 2002). Like other sexually selected traits, the adaptive phenotypic plasticity exhibited in acoustic signals has important consequences for male reproductive success, because males must trade off competing demands to attract mates, repel rivals and avoid predators (Bertram, Harrison, Thomson, & Fitzsimmons, 2013). Energetic costs are a particularly important factor in signalling plasticity: acoustic communication is energetically demanding (Bailey, Withers, Endersby, & Gaull, 1993; Gerhardt & Huber, 2002), increasing an individual's metabolic rate while singing (Prestwich, 1994; Stevens, & Josephson, 1977) and even at rest (Reinhold, 1999), and females often prefer more energetically costly signals (Ryan & Keddy-Hektor, 1992). Therefore, energetically costly signals can honestly reflect the quality of the sender (Grafen, 1990) with males in poorer condition producing less attractive signals compared to males in better condition (Podos & Patek, 2015). At the individual level, the trade-off between being an efficient signaller and coping with the costs of expensive acoustic features (Mowles, Cotton, & Briffa, 2011; Reichert & Gerhardt, 2012; Smith & Harper, 2003), increases with the strength of the competition (Johnstone, 1997; Wilgers & Hebets, 2015). A male's ability to respond to competition may also reflect its quality, because competition requires the production of even more expensive signals (Parker, 1974; Reichert, 2014).

In Orthoptera, acoustic signalling is a fundamental behaviour to attract conspecific females and to compete against rivals (Gerhardt & Huber, 2002; Robinson & Hall, 2002). A high-quality signaller may communicate a superior body condition, a characteristic that is likely to be relevant for both female choice and male-male competition, via its acoustic signalling performance (Schatral & Bailey, 1991; Wilgers & Hebets, 2015). Females may evaluate the qualities of a potential mate using intensity, frequency and/or the temporal structure of male songs (Schatral & Bailey, 1991). They are mostly attracted by signals with high intensity, long duration and high repetition rate (Gerhardt & Huber, 2002; Greenfield, 2002) and usually choose larger or heavier competing males (reviewed in Gwynne, 2001; Lehmann, 2012). Acoustic signals may vary between individuals (Bailey, 1985; Hedrick, 1986; Latimer, 1981a; Samways, 1976; Simmons, 1988). Factors causing this inter-individual variation may

be internal such as body mass, body size and energetic condition of the signalling male, or external such as the number, quality and proximity of competitors in the environment. Body size affects both the intensity and frequency of the acoustic signal as larger males produce louder songs at a lower fundamental frequency (Bailey & Thiele, 1983; Forrest, 1983; Latimer & Schatral, 1986). The social environment is an important external environmental factor because Orthopterans signal for mates in choruses (Greenfield, 1994) in which males engage in signal competition with their neighbours and males may vary their call characteristics as a consequence of the vocal activity of neighbours (reviewed by Greenfield, 2005). A male signaller might hear the calls of conspecific rivals, gaining acoustic information about size or vigour, fighting ability or ability to attract females (Busnel, 1967; Greenfield & Shaw, 1983; Latimer & Schatral, 1986; Latimer, 1981b; Römer & Bailey, 1986). Notably, male crickets and bushcrickets can modify their chirp interval and chirp duration when rivals are signalling (Brush, Gian, & Greenfield, 1985; Dadour, 1989; Jones, 1974; Samways, 1976; Shaw, 1975). Chorusing behaviour might present a cooperative effect: choruses of many hundreds of individuals even without strict synchronization might increase the calling space (Krobath, Römer, & Hartbauer, 2017) and reduce the individual predation pressure including that of acoustic-orienting parasitoid flies (Lakes-Harlan & Lehmann, 2015; Lehmann & Heller, 1998).

Remarkably little work has been done in orthopterans to test how different internal and external sources of variation may cause the plasticity of the acoustic features produced during song competition. Here, we studied contests between males of different body mass in combination with manipulation of the social environment (the intermale rival distance) to investigate how both may affect the acoustic signal behaviour of males of the bushcricket species *Poecilimon ampliatus* (Orthoptera: Tettigoniidae: Phaneropterinae) during acoustic contests. As male body mass is an important factor for female choice (Lehmann & Lehmann, 2008) and reflects a male's condition (Lehmann & Lehmann, 2009), we examined the role of this factor during song contests between males of different body mass. Moreover, since close-range acoustic competition occurs naturally in this species which aggregates in high-density populations (Lehmann, & Lehmann n.d.), we evaluated the signal production activity under varying competition, with different intermale rival distances. First, we compared the acoustic signalling behaviour of heavy and light males, both in isolation and under four competition treatments: we predicted a significant signalling discrepancy between the two body mass classes both in competition and in isolation. Heavy males might always signal better than the light rivals. Second, we tested whether male signalling activity is plastic with respect to the distance to a rival by comparing song characteristics across different rival distances. If intensity of competition increases when the rival comes closer, we predicted that both heavy and light males would show a plastic response to the variation in the social context, by increasing signal production in terms of sound duration, rate and total amount of time spent signalling, when hearing rivals close by.

3.3. Methods

3.3.1 Study species

Poecilimon ampliatus is a flightless species in a genus distributed predominantly in South-eastern Europe. Despite an ancestral bidirectional communication system with males signalling and females responding, *P. ampliatus* belongs to those species that secondarily reverted to a unidirectional system; only males produce acoustic signals and females locate them by phonotaxis (Heller & von Helversen, 1993). Males usually start signalling four days after moulting to adulthood and their song consists of subunits, called verses, which are separated by silent pauses (Heller, 1988; Heller & Lehmann, 2004) (Fig. 3-1).

Male nymphs were collected near the village of Gabrče (45°42'40" N, 14°01'05" E) in Slovenia. They were individually caged in 200-ml marked boxes (*Drosophila* rearing boxes; Greiner Bio-one GmbH) and transferred to the laboratory, at the Institute of Biology at the Humboldt University Berlin, where the experiments were performed. The animals were reared under an *ad libitum* feeding regime with fresh leaves of *Taraxacum officinale* and flower pollen (Lehmann & Lehmann, 2016), a temperature of 24°C ± 1°C and a light : dark cycle at 15:9 h similar to the natural conditions.

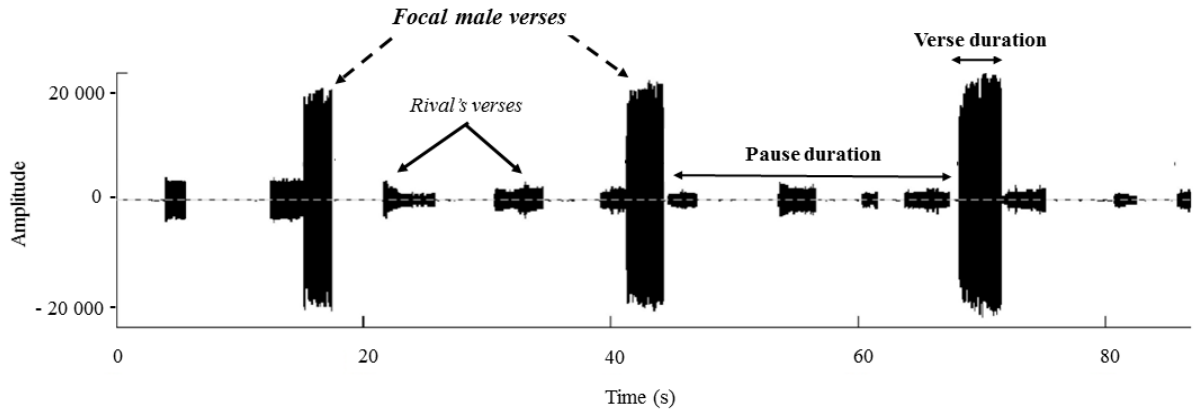

Figure 3-1. Oscillogram representing the temporal acoustic features of *P. ampliatus*. During a sampling period of 90 s, the focal male produced three verses separated by two pauses. A total of 12 verses of the rival male were detected as background noise. Verse and pause duration of the focal male are indicated. Sample rate 96 kHz, amplitude threshold = 30 dB, minimum verse duration = 0.5 s.

3.3.2 Experimental Design

The experiments involving acoustic interactions between competing males were conducted in the laboratory during two breeding seasons (May - June 2014, May - June 2015). Acoustic interactions between light and heavy males were recorded in an isolated acoustic chamber (2 m^2) with temperature and humidity in the chamber kept constant.

All animals tested were virgin and recorded at 8 days after final moult. We used the male's body mass as a proxy for body condition. Right before starting the experiment, we weighed all males

(KERN ALT 100-5AM; accuracy ± 0.01 mg) to create 28 competing pairs (= 56 individuals), composed of one heavy and one light rival male. Heavy males (mean ± SD = 430.82 ± 36.14 mg) were around 100 mg heavier than light males (mean ± SD = 338.29 ± 36.20 mg), leading to a significant body mass difference (ANOVA: $F_{1, 54} = 91.65$, $P < 0.001$). A commonly used body size indicator (hind femur length) is only weakly correlated with body mass as shown in previous studies within the genus *Poecilimon* (e.g. Lehmann & Lehmann, 2009) and therefore omitted from the analysis.

Calling males were placed individually on a foam pad and covered by a wire-mesh enclosure to prevent them from escaping. In addition to this physical isolation, males were kept in acoustic isolation before each trial. Once placed in the chamber, males were allowed to acclimatize for 5 min before starting the experiment.

For baseline data acting as an intra-individual control, each male was recorded in isolation before being tested in competition. To simulate different intensities of the song contest, the two males (light and heavy) were tested at four rival distances: 10 cm, 60 cm, 180 cm and 240 cm. These distances were selected based on naturally occurring male-male distances, ranging from 20 individuals/ m2 to sparse populations with 2-3 m between males in Slovenia (Lehmann, & Lehmann, n.d.). At every distance, males were recorded five times (‘trials’) for 90 s per trial. To avoid habituation to the competition situation males were acoustically isolated between the trials for 30 min. To simulate an escalating contest, the recording started at the furthest distance (240 cm), continued with the next closest one, and ended at the highest level of competition at 10 cm.

3.3.3 Recordings

Male songs were recorded (Fig. 1) using a digital audio recorder (EDIROL R-44, Roland Corporation, Japan; 96 kHz sampling rate; 16 bit); the digital stereo audio file was stored as a wav-file. To obtain separate recordings of each male's calls, two directional microphones (Sennheiser MKH 40-P48, Sennheiser Electronic, Wedemark, Germany) were placed on a tripod 15 cm above each test male. For the analysis, the stereo file was split into two mono-channels, each associated with one male.

3.3.4 Analysis of Temporal Song Characteristics

The time domain of a male's song was analysed for four parameters. The duration of the verse was measured as well as the duration of the silent pause between verses (Fig. 3-1). The verse rate was calculated as the number of verses during the recording period of 90 s. A derived measurement is the duty cycle, which is the total time a male spent calling relative to the time spent silent, equalling the summed duration of all verses, divided by the total recording time.

The software packages tuneR (Ligges, Krey, Mersmann, & Schnackenberg, 2016) and seewave (Sueur, Aubin, & Simonis, 2008) in R were used for automated detection of verses (Fig. S3-1). In some cases, owing to the high level of temporal overlap of the subject males' calls, the temporal song characteristics were measured manually using Audacity software version 2.0.5

(https://www.audacityteam.org). Audacity was also used to evaluate the accuracy of values measured automatically by seewave. In total, there were up to 25 recordings for each pair of rival males, five replicates in isolation and five replicates under competition at four different rival distances. However, the full set of 25 recordings could not be obtained for all males, as not all males sang continuously when tested in isolation and in the low competition situation (at 240 cm). Therefore, recordings with only one signalling male were excluded.

3.3.5 Statistical Analysis

All statistical analyses were performed with R v. 3.2.3 (R Core Team, 2015) and R-Studio v. 1.1.383 (R Studio Team, 2016). We used separate liner mixed models (lme4 package: Bates, Mächler, Bolker, & Walker, 2015) for each acoustic variable, fitted using restricted maximum likelihood estimation (REML), to test for signal adjustment in acoustic competition due to male body mass and rival proximity. Following the suggestions by Lo and Andrews (2015) and the Akaike information criterion (AIC; Akaike, 1998) approach, we used a linear mixed model (LMM) with Gaussian distribution and log-transformed dependent variables because it gave the best model fit, resulting in the lowest AIC value and best diagnostic plots (Table S3-1, Fig. S3-2).

For each acoustic variable, we considered in our model the terms 'body mass' and 'rival distance' as fixed effects. The interaction term 'body mass * rival distance' was also included. With a likelihood ratio test, we compared three models with different random effect terms against one another, using the 'anova' function from the 'stat' package (Table S3-2). From the P value obtained by comparing these models, we tested whether changing the random effect terms influenced the predictive ability of the model (Bates *et al.,* 2015; Pinheiro & Bates, 2000). The first model (1) presented only the random slopes for 'pairs' specified, so that both the effect of variation in pairs on the intercept (random intercept) and the effect of variation in rival distance (random slope) are included and the variances for the five levels of rival distance are allowed to be different. In other words, it models potential between-pair heteroscedasticity with respect to rival distance values. The second model (2) included the previously described random slope and the random factor 'male ID' nested within 'pairs', because in our experimental design each experimental pair was an exclusive pair of males (one heavy and one light). The third model (3) included the previous random effect terms plus a random term for 'trials', to check whether including the variance of the five replicates might affect the model fit. While the nested random factors significantly increased the fit of the model and decreased the AIC value, this was not the case when the random factor 'trials' was added (Table S3-2). Consequently, we adopted for the subsequent analyses the best model (2), which included only the slope and the nested random factors. We calculated P values for fixed effects by performing a type III ANOVA on the model, with the function 'anova' from the package 'lmerTest' (Kuznetsova, Brockhoff, & Christensen, 2015). The evaluation degrees of freedom are based on the Satterthwaite

approximation. The post hoc analysis on the contrast was performed using the 'step' function from 'lmerTest' (Kuznetsova *et al.,* 2015).

3.4. Results

Male *P. ampliatus* in competition adjusted their acoustic signalling activity depending on their body mass and the perceived competition strength based on rival distance (Figs. 3-2 and 3-3). The results obtained from the best model found for every acoustic variable (Table 3-1) show that male body mass and rival distance were the two main factors affecting the males' acoustic variables investigated in this study, excluding verse rate. The effect of male body mass on verse and pause duration and duty cycle was stronger than the effect of the rival distance (Table 3-1). For all acoustic characters, except the duty cycle, the interaction effect between body mass and rival distance was not significant.

Table 3-1. Acoustic parameters of *P. ampliatus* males.

Acoustic characters	**Num. df**	**Den. df**	***F***	***Pr (>F)***
Verse duration				
Body mass	1	31.11	23.77	**< 0.001**
Rival distance	4	21.72	3.80	**0.017**
Body mass*Rival distance	4	649.75	2.00	0.094
Duty cycle				
Body mass	1	35.51	32.39	**< 0.001**
Rival distance	4	18.82	5.67	**0.003**
Body mass*Rival distance	4	657.31	3.24	**0.012**
Pause duration				
Body mass	1	36.59	17.58	**< 0.001**
Rival distance	4	19.78	9.78	**< 0.001**
Body mass*Rival distance	4	650.64	1.00	0.405
Verse rate				
Body mass	1	43.42	2.815	0.100
Rival distance	4	28.49	1.8248	0.152
Body mass*Rival distance	4	664.30	0.853	0.492

Acoustic parameters were tested for the influence of male body mass, rival distance and their interaction. Significant probabilities of models performed for every acoustic variable with the Satterthwaite approximation are in bold. Number of observations = 759, number of pairs = 28.

In contrast to our first prediction, heavy males did not differ from their light rivals for all signal characteristics in all the distance treatments. In isolation, verse duration (Fig. 3a), duty cycle (Fig. 3c) and verse rate (Fig. 3d) of heavy and light males did not differ significantly. In this condition, heavy

males signalled on average with 2 s longer verses and only with a 6% higher duty cycle than light males (Fig. 2a).

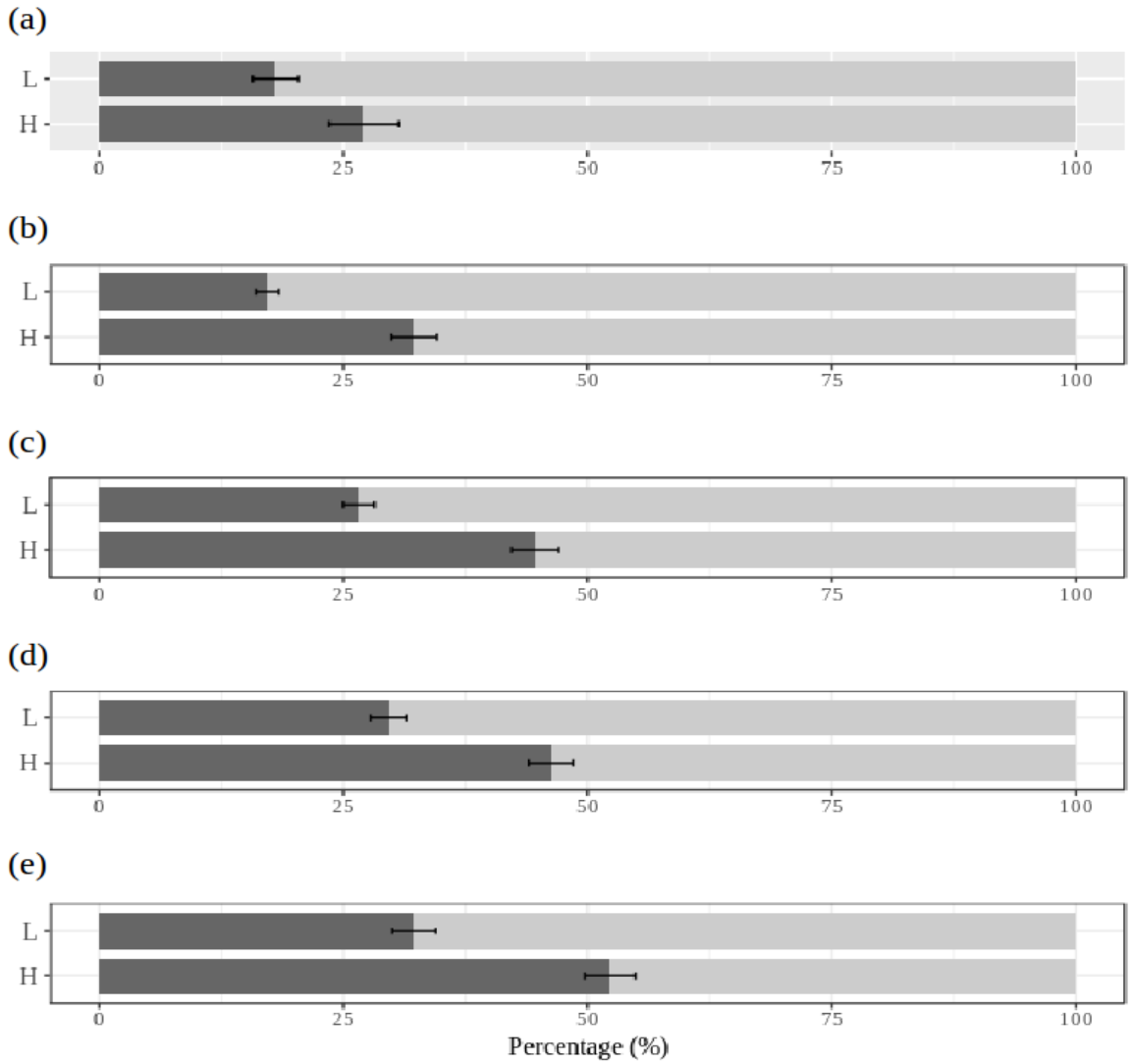

Figure 3-2. Relative time spent signalling (duty cycle), normalized to 100% stacked bar plot, reporting verse (dark grey) and pause duration (light grey) separated by rival distances. The percentage of male signalling is shown in (a) isolation and in competition at decreasing rival distances: (b) 240 cm; (c) 180 cm; (d) 60 cm; (e) 10 cm. In each stacked bar plot, the upper bar reflects light males (L) and the lower heavy males (H). Standard error bars are shown for verse duration.

Conversely, heavy males signalled more intensely than light males in competition and the effect of male body mass was stronger at the closest rival (Fig. 2e) distances than at the furthest (Fig. 2b). At intermediate and high competition levels, heavy males signalled with longer verses (Fig. 3-3a), shorter pauses (Fig. 3-3b) and higher duty cycles (Fig. 3-3c) than light rivals. This difference in acoustic signalling activity between heavy and light males is highly significant for verse duration at all three rival distances: 10 cm (*t* test: $t_{64.2} = 5.27$, $P < 0.001$), 60 cm (*t* test: $t_{64.6} = 4.23$, $P < 0.001$) and 180 cm (*t* test: $t_{62.8} = 5.23$, $P < 0.001$). Similarly, the duty cycle of heavy and light males differed for a rival

distance of 10 cm (*t* test: $t_{69.7} = 6.44$, $P < 0.001$), 60 cm (*t* test: $t_{70.1} = 4.77$, $P < 0.001$) and 180 cm (*t* test: $t_{68.3} = 5.92$, $P < 0.001$). As the rival distance increased up to 240 cm, the effect of male body mass on the difference between signals became weaker (Fig. 3-2b). However, heavy males still signalled with significantly longer verses (*t* test: $t_{109} = 2.35$, $P < 0.05$) and higher duty cycles than light males (*t* test: $t_{118.4} = 3.38$, $P < 0.05$). The signalling activity maintained even at the lowest competition level, with the rival at 240 cm, was due to the increased verse rates recorded mostly in heavy individuals (Fig. 3-3d). Furthermore, our results are consistent with the prediction based on our second hypothesis. The males' signalling activity increased with competition strength and therefore with the reduction of the distance to the rival. However, our prediction is not completely fulfilled as the distance to the rival affected only heavy males. In this group, we found a significant difference in signal characteristics between the isolation and the competition situations (Figs. 3-2 and 3-3). In fact, heavy males in isolation signalled only one-quarter of the time (Fig. 3-2a) but increased their acoustic signal production under competition (Figs. 3-2b-e and 3-3). Between isolation and the weakest competition at 240 cm, heavy males showed significant increases in duty cycle (*t* test: $t_{61.7} = -2.12$, $P < 0.05$; Fig. 3-3d), while there was no variation between these two contexts in verse duration (Fig. 3-3a). Light males had very similar values when signalling alone or versus heavy rivals at 240 cm for all the song parameters studied (Fig. 3-3a-d).

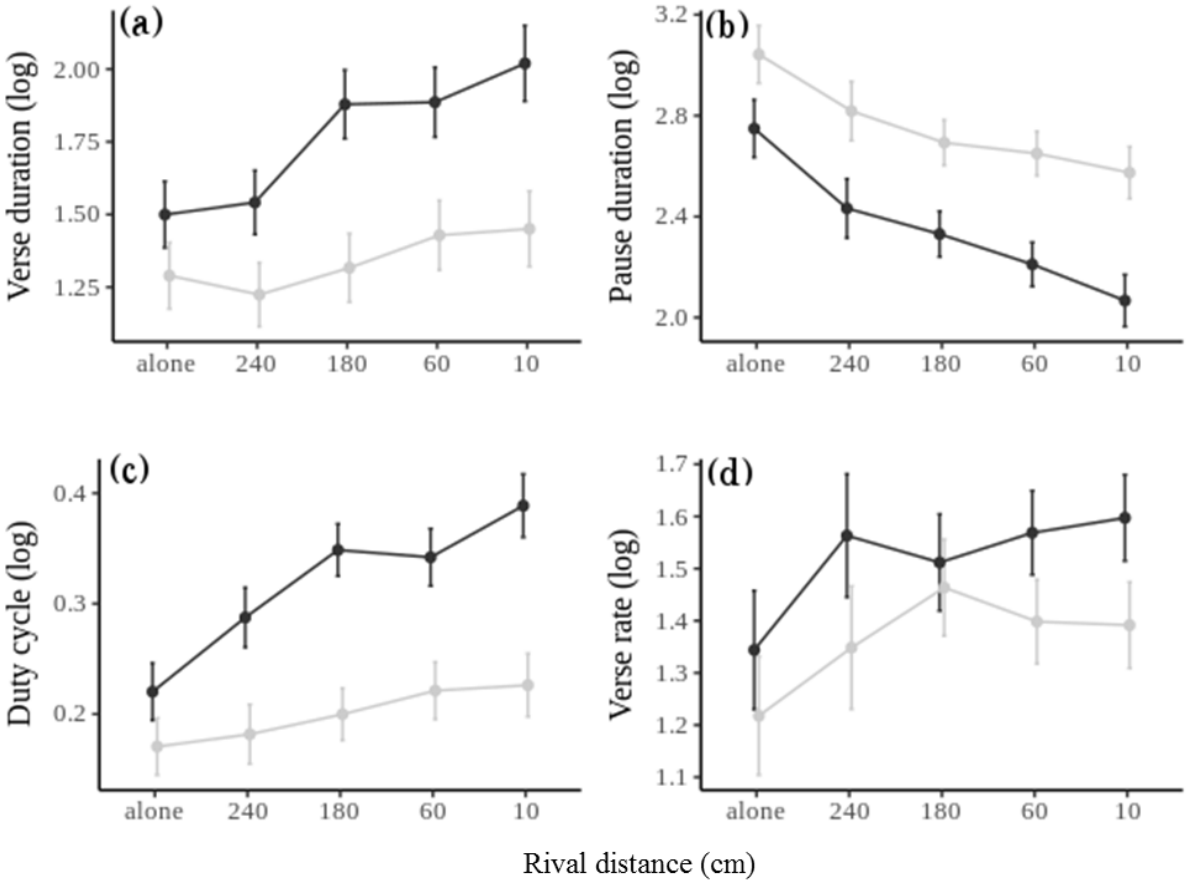

Figure 3-3. Acoustic characters of *P. ampliatus* males, given in log-transformed values, tested in isolation (alone) and at four intermale rival distances, decreasing from 240 cm to the highest competition at 10 cm. (a) Verse duration; (b) pause duration; (c) duty cycle; (d) verse rate. Means ± SE are given for heavy (black) and light males (light grey).

Under more intense competition due to shorter rival distances, heavy males further increased their acoustic activity (Fig. 3-2c-e, 3-3). When the light rival was at 180 cm, heavy males nearly doubled their duty cycle (Fig. 3-3c) which was significantly higher than in isolation (*t* test: $t_{54.2} = -4.02$, $P < 0.001$) or at 240 cm (*t* test: $t_{33.5} = -2.13$, $P = 0.040$). The duty cycle at a rival distance of 60 cm was also significantly higher than in isolation (*t* test: $t_{53.9} = -4.03$, $P < 0.001$). However, between 60 and 240 cm, the duty cycle variation was marginally significant (*t* test: $t_{22.3} = -1.83$, $P = 0.081$). At 10 cm, we found the highest increase in the duty cycle compared with isolation (*t* test: $t_{50.2} = -5.19$, $P < 0.001$) and with the light rival at 240 cm (*t* test: $t_{65.8} = -3.59$, $P < 0.001$). Verse duration increased on average by 5 s in heavy males at rival distances of 180 (Figs. 3-2c) and 60 cm (Fig. 3-2d) compared to 240 cm (Fig. 3-2b) or signalling in isolation (Fig. 3-2a). All three comparisons for heavy males revealed a significant increase in verse duration compared to isolation over the rival distances of 180 cm (*t* test: $t_{72.7} = -3.06$, $P = 0.003$), 60 cm (*t* test: $t_{58.5} = -3.00$, $P = 0.004$) or 10 cm (*t* test: $t_{52.3} = -3.93$, $P < 0.001$) and between a rival at 240 cm in comparison to 180 cm (*t* test: $t_{47.9} = -2.74$, $P = 0.009$), 60 cm (*t* test: $t_{47.2} = -2.75$, $P = 0.008$) and 10 cm (*t* test: $t_{29.3} = -3.60$, $P = 0.001$). In contrast, light individuals did not

significantly modify their verse duration across different rival distances. During competition heavy males also increased verse durations (Fig. 3-3a). Notably, between the weakest (rival distance = 240 cm) and the strongest competition (rival distance = 10 cm) verse duration increased by 8 s (Fig. 3-3a).

3.5. Discussion

In our study, acoustic plasticity was linked to both the intrinsic body condition of the signalling individuals and an extrinsic or environmental factor (rival distance). As is common for sexual signals (Andersson, 1994), male acoustic signalling was expressed in a condition-dependent manner and varied with competition due to rival presence. In *P. ampliatus* males both verse duration and duty cycle are good predictors of body condition. Heavy males invested more in acoustic signalling activity, by producing longer verses and higher duty cycles when competing against a light rival.

Contrary to the first prediction, we found only minor differences in acoustic traits between heavy and light males signalling in isolation. This is congruent with a previous study in another bushcricket species (Hartbauer, Kratzer, & Römer, 2006), in which fitness differences due to feeding regimes were not reflected in chirp rates in isolated signalling males. Given the similarity in acoustic production between light and heavy males when isolated, females might have little chance of judging the quality of a single male. In fact, in a previous study of a congeneric Poecilimon species females accepted all males in single-choice experiments regardless of their body mass (Lehmann & Lehmann, 2008) and the nutritional benefit they might gain by mating with heavier males (Lehmann & Lehmann, 2009). Males might avoid additional signalling costs when there is no perceived need for it (i.e. when there are no competitors), and instead produce a minimally sufficient signal. In competition, heavier males always signalled more intensely than their lighter rival. Extreme conditions of competition maximized the difference in acoustic characteristics between heavy and light males. Under high acoustic competition (distance from the rival: 10 cm), heavy males sang with an especially high effort compared to their lighter rivals. However, heavy and light males did not differ in verse rate.

In crickets, song duration and duty cycle are considered honest indicators of body condition (Gray, 1997; Hack, 1997; Scheuber, Jacot, & Brinkhof, 2003; Shackleton, Jennions, & Hunt, 2005; Souroukis & Cade, 1993) and ensiferan males in poor body condition signal acoustically with less effort (Holzer, Jacot, & Brinkhof, 2003; Ritchie, Sunter, & Hockham, 1998; Simmons *et al.,* 1992; Wagner & Hoback, 1999). Through song structure, males convey important information about their body condition (Brown, Smith, Moskalik, & Gabriel, 2006; Lehmann, 2007) and this prevents poor quality males using deceptive signals (Polnaszek & Stephens, 2014). These results imply important consequences for male fitness in the wild, because females assess potential mates based on their song characteristics (Schatral & Bailey, 1991). For instance, in *Poecilimon zimmeri* heavier males were highly preferred by females for mating over lighter males (Lehmann & Lehmann, 2008).

Acoustic signalling during competitive interactions is known to be costly in many taxa (Briffa & Elwood, 2001). The energetic costs of calling can be particularly high in species where females tend

to choose those males that call for longer, at higher rates or louder (Doubell et al., 2017; Erregger *et al*., 2017). Indeed, increased song production in bushcrickets requires an increased metabolic rate (reviewed by Lehmann, 2012). Signal production appears to honestly convey information about the male's quality, since individuals reared under poor dietary conditions or infected by acoustically orienting flies decreased signalling activity and were less attractive for females (Lehmann & Lehmann, 2006). However, the exact physiological costs of signalling in *P. ampliatus*, which might affect the production of these costly sexual signals, need to be disentangled in further studies.

Our results highlight that heavy males increased their signal production as the intermale distance decreased. The critical rival distance for heavy males was at 180 cm, at which there was a drastic increase in verse duration and duty cycle, in comparison to lower levels of competition. This change in the male's signalling effort, depending on the perceived competition level, is consistent with previous studies, which showed that individuals adjust their signalling effort based on the social environment (Bretman *et al.,* 2011; Greenfield, 2005, 2015; Greenfield, Marin-Cudraz, & Party, 2017; Krobath *et al*., 2017; Rebar & Rodríguez, 2016). For example, in an experiment performed in tree frogs by Reichert and Gerhardt (2012), increasing competition (via decreased rival distance) led males to increase their call duration and duty cycle, reaching the maximum when the rival was closest.

Interestingly, heavy males could modify verse duration and duty cycle independently of one another across all the different competition conditions, showing the higher adaptive plasticity of these individuals when in competition with weaker rivals. Heavier individuals might have more resources available for reacting to environmental variation, increasing their signalling more efficiently than lighter males in line with a cost of plasticity (Thomas, Sih, & Wilson, 1998).

The plasticity due to competition clearly reflects the males' ability to hear rivals. The dominant frequency of the songs of *P. ampliatus* males is between 20 and 40 kHz (Heller & Lehmann, 2004; Heller, 1988) and the power output at 10 cm is around 120 dB SPL in similar species of the same genus with a comparable body size (Lehmann & Heller, 1998). Thus, if we consider that a 40 kHz signal attenuates about 1 dB/m of its power in air (Forrest, 1994) and that the hearing sensitivity threshold of this species is mainly above 70 dB SPL at this frequency (Lehmann, Strauß, & Lakes-Harlan, 2007; Strauß, Lehmann, & Lehmann, 2014), we can assume that males should be able to perceive the signal up to 40 m. In our study design, the rival's song was easily perceptible by males, explaining why heavy males competed up to 240 cm. The reduced calling activity found in lighter males cannot be related to reduced hearing capacities (Lehmann et al., 2007; Strauß *et al.,* 2014), but clearly represents a behavioural strategy adopted by light individuals. As heavier males might have larger bodies and in turn bigger hearing organs, it might be that these males have an improved acoustic perception capacity as well. In a previous cross-species comparison study within the genus (Strauß *et al*., 2014), males' hearing organs were shown to scale positively with body length in bidirectionally communicating species of this genus, but not in species with unidirectional communication, such as *P. ampliatus*, where the effect of body length on these organs was surprisingly weak.

At the weakest level of competition, with the rival spaced at 240 cm, heavy males signalled almost with the same duration of verses as they did in isolation, but with an increased duty cycle. Thus, heavier males signalled intensively even at greater intermale distances, thereby competing over larger space and increasing their chances of passively attracting females (Parker, 1983; Robinson & Hall, 2002; Ronacher, 2016).

Nevertheless, it is important to underline that we used caged males, preventing them from exploiting alternative strategies as found in other Orthoptera (Arak & Eiriksson, 1992; Bailey & Field, 2000; Farris, Forrest, & Hoy, 1997; Forrest & Green, 1991). In crickets, males tend to switch to silent searching at higher densities (Hissman, 1990; Simmons, 1986), with the frequency of satellite behaviour increasing with male density (Cade & Cade, 1992). In nature, males of *P. ampliatus* do not engage in physical fights and do not defend territories (G. U. C. Lehmann, personal observation). However, they occur in densities up to 20 individuals/m2, so moving away from a single male is very likely to reduce the distances to other males. The lighter males in our experiments kept on signalling, not switching to an alternative behaviour. Much more remains to be studied in a systemic way to understand how males adapt to such a complex and dynamic acoustic environment.

3.6. Conclusions

Through examining the acoustic signal behaviour of the bushcricket *P. ampliatus*, we showed that body condition affects male signalling activity and this effect is amplified with the intensity of the contest. Hence, signalling males apparently evaluate their rival's distance, a form of activational behavioural plasticity (Snell- Rood, 2013) that presumably allows males to manage the resources invested during acoustic signalling. Bushcrickets signalling in aggregations have ample opportunities to gather information about other group members and signalling males modify their activity depending on the presence and proximity of neighbouring rivals (Balakrishnan, 2016; Greenfield, 2015; Greenfield, Esquer-Garrigos, Streiff, & Party, 2016; Marin-Cudraz & Greenfield, 2016; Murphy, Thompson, & Schul, 2016). Acoustic contests seem to play an important role in this species, in shaping a male's signalling strategy and plasticity in the natural chorus, where more than two competitors sing simultaneously.

3.7. Acknowledgement

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design and on the manuscript. Special thanks to Arne Lehmann, Camille Desjonquères and Nadja Wulff for useful discussions.

3.8. Supplementary Information

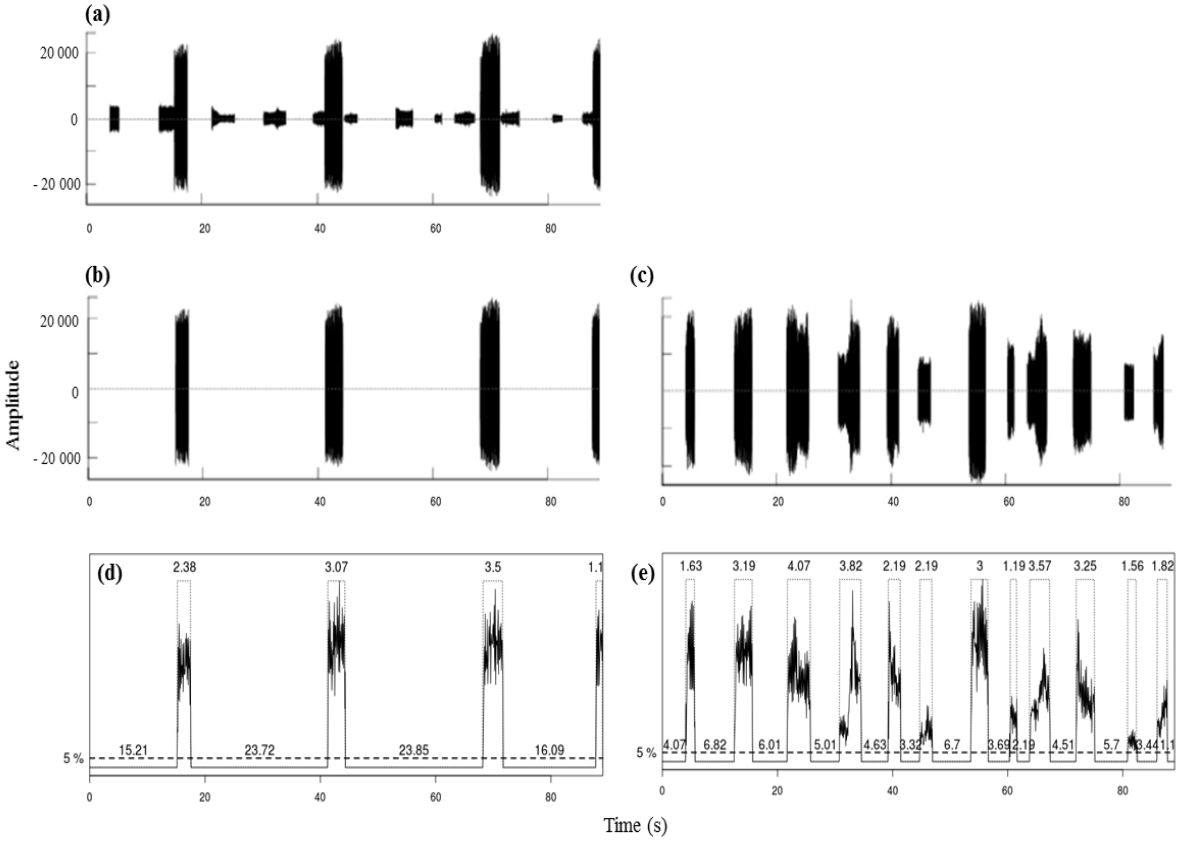

Figure S 3-1. Automatic pre-processing of audio recordings of *P. ampliatus* males using the software packages tuneR and seewave in R. (a) Step 1: males recorded in stereo; (b) step 2: audio from left channel with only light male signal; (c) step 3: audio from right channel with only heavy male signal; (d) step 4: timer function on left channel; (e) step 5: timer function on right channel.

Table S 3-1. Linear (LMM) and general (GLMM) mixed model selection.

Distribution Verse duration (DV)	Link function	AIC	BIC
LMM			
Gaussian (Raw DV)	Identity	5347.5	5477.2
Gaussian (Log (DV))	**Log**	**1303.5**	1433.2
Gaussian (-1000/ DV)	Inverse	9245.6	9375.3
GLMM			
Gamma (Raw DV)	Identity	3520.4	3650.1
Gamma (Raw DV)	Log	3752.4	3882.0
Gamma (Raw DV)	Inverse	3747.4	3877.1
Inverse Gaussian (Raw DV)	Identity	3851.7	3981.4
Inverse Gaussian (Raw DV)	Log	3465.6	3595.3
Inverse Gaussian (Raw DV)	Inverse	4048.7	4169.1

AIC and BIC indices of model fit comparing LMMs and GLMMs of different distribution and link assumptions. The dependent variable (DV), is tested with LMM and GLMM models. In LMM the DV is expressed raw, log (Log (DV)) and inverse transformation (-1000/DV). In GLMM the DV is always considered raw. In bold, is the lowest AIC value (Gaussian, log – link).

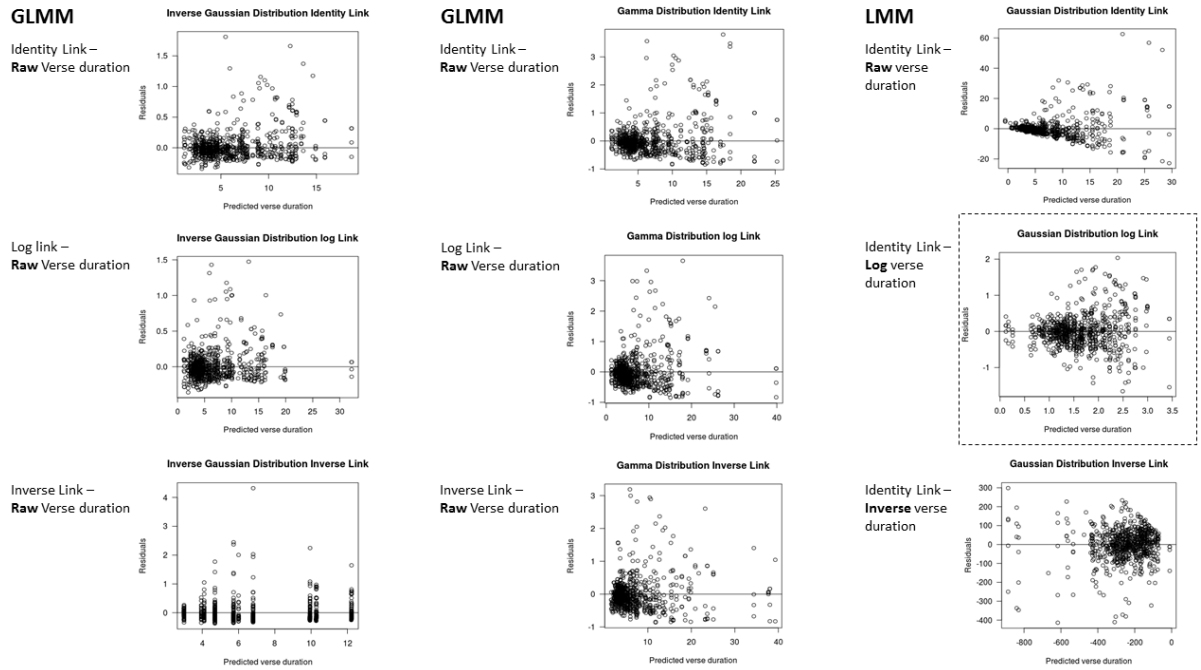

Figure S 3-2. Plots of the residuals over predicted verse duration. Each column of plots represents a different assumption for the distribution of verse duration (inverse Gaussian, gamma and Gaussian), and each plot represents the relationship between the predictors and verse duration (identity, logarithmic and inverse link function). Dashed box highlights the best distribution: Gaussian log transformation of verse duration.

Table S 3-2. Random term model's comparison for each acoustic characteristic of *P. ampliatus* males.

Model	Random factors	Syntax	*Df*	AIC	BIC	logLik	deviance	χ^2	*Df* (χ^2)	*Pr* (>χ^2)
Verse duration										
1	Random slope	(1 + Rival distance | Couple)	26	1367.17	1487.60	-657.58	1315.17			
2	Random slope + nested factor	(1 + Rival distance | Couple) + (1 | couple / male_id)	28	**1303.49**	1433.19	-623.74	1247.49	67.68	2	**< 0.001**
3	Random slope + nested factor + crossed factor	(1 + Rival distance | Couple) + (1 | couple / male_id) + (1| trials)	29	1305.45	1439.77	-623.72	1247.45	0.04	1	0.834
Duty cycle										
1	Random slope	(1 + Rival distance | Couple)	26	1555.32	1675.75	-751.66	1503.32			
2	Random slope + nested factor	(1 + Rival distance | Couple) + (1 | couple / male_id)	28	**1442.89**	1572.59	-693.45	1386.89	116.43	2	**< 0.001**
3	Random slope + nested factor + crossed factor	(1 + Rival distance | Couple) + (1 | couple / male_id) + (1| trials)	29	1444.79	1579.11	-693.39	1386.79	0.10	1	0.747
Pause duration										
1	Random slope	(1 + Rival distance | Couple)	26	1418.59	1539.03	-683.30	1366.59			
2	Random slope + nested factor	(1 + Rival distance | Couple)+ (1|couple / male_id)	28	**1294.17**	1423.87	-619.08	1238.17	128.43	2	**< 0.001**
3	Random slope + nested factor + crossed factor	(1 + Rival distance | Couple) + (1 | couple / male_id) + (1 | trials)	29	1296.09	1430.42	-619.04	1238.09	0.08	1	0.776
Verse rate										
1	Random slope	(1 + Rival distance | Couple)	26	1305.83	1426.26	-626.92	1253.83			
2	Random slope + nested factor	(1 + Rival distance | Couple)+ (1 | couple / male_id)	28	**1210.31**	1340.00	-577.15	1154.31	99.52	2	**< 0.001**
3	Random slope + nested factor + crossed factor	(1 + Rival distance | Couple) + (1 | couple / male_id) + (1 | trials)	29	1212.31	1346.64	-577.15	1154.31	0.00	1	1.000

Comparison between models fitted using the restricted maximum likelihood (REML) estimation. AIC: Akaike information criterion; BIC: Bayesian information criterion. Significant P values and lowest AIC values are in bold. Model's random effects definition: random slope model 1 = (1 + RD | Pair), random slope + nested factor model 2 = (1 + Rival distance | Pair) + (1| pair / male_ID), random slope + nested factor + crossed factor model 3 = (1 + Rival distance | Pair) + (1| pair / male_ID) + (1| trials).

Chapter 4. Body condition affects plasticity in acoustic signalling of male bushcrickets in socially heterogeneous choruses

Based on: Anichini M., Rebrina F., Reinhold K., Lehmann G.U.C. 2018. "Body condition affects plasticity in acoustic signalling of male bushcrickets in socially heterogeneous choruses". Paper submitted to *Animal Behavior* - Elsevier

4.1. Abstract

In many animal species, males' acoustic signals are produced to attract a conspecific female. In a chorus, where multiple signallers are present, males also act as receivers and they actively adjust their acoustic production depending on the presence of mating rivals. In competition, plasticity in signal features might be advantageous and males could tune their signalling activity to the intensity of contest. However, several limits and costs might constrain the ability to exhibit plastic behaviour and inter-individual variation in plasticity can occur.

In this field study, we examined the influence of body mass and social environment on the signal expression of bushcricket males (*Poecilimon veluchianus veluchianus*). We compared the variation in the acoustic response of heavy and light males, testing them against rivals of different body mass and number. We found that only heavy focal males displayed significant plasticity in their signalling behaviour. Under the highest competition pressure (against two heavy rivals) they signalled with the minimum effort, using shorter verses with a reduced number of syllables than when competing against one heavy rival or two light rivals. In competition with light rivals, heavy males signalled more when facing two rivals than only one. In contrast, light focal males did not vary their signal features, which remained almost constant through different competition levels. These results indicate that body mass and social context drive inter-individual variation in the capacity of *P. v. veluchianus* males to adjust their acoustic features to competition levels. We discuss several possible explanations for these findings and provide suggestions for future work to examine evolutionary aspects of socially induced plasticity in sexually selected acoustic features.

4.2. Introduction

Environmental variation provokes a variety of responses in organisms, and behavioural plasticity is an important, and often the first, response to changes in environmental conditions (Huey, Hertz, & Sinervo, 2003; West-Eberhard, 2003). The ability to produce an adaptive plastic response should be favourable, since it allows individuals to survive and persist in altered environments (Price, 2006; Robinson & Dukas, 1999), and to increment their fitness (Snell-Rood, 2013; Sullivan-Beckers & Hebets, 2014). However there might be several types of measurable costs and limit that might potentially constrain the evolution of plasticity (DeWitt, Sih, & Wilson, 1998a; Pigliucci, 2005; Van Tienderen, 1991). Even though plasticity can be costly "*per se*", only because an organism possess the ability to be plastic, examples of potential costs are found in the reduced reproduction and grow, anomalies in developing the plastic response and performing the alternative behaviours, as well as, in the maintenance of the "machinery" that provides plasticity (DeWitt, Sih, Wilson, 1998). Limits of plasticity could be potentially identified in the range of development, information-reliability, epiphenotype, plasticity-history and ecology (DeWitt, Sih, Wilson, 1998). According to theoretical models, natural selection will favour phenotypic responses that balance cost avoidance and resource acquisition (Ernande & Dieckmann, 2004). Such costs and limits on behavioural plasticity appear to vary substantially between individuals within a population (Briffa, Rundle, & Fryer, 2008; Dingemanse & Wolf, 2013). In nature, not all organisms always exhibit the "optimal" plasticity in all their traits (DeWitt, Sih, & Wilson, 1998; Via et al., 1995) and some individuals are more responsive to changes in the environment than others (Réale & Dingemanse, 2010). Few empirical studies have reported variation in the plasticity level among populations of the same species (Baythavong & Stanton, 2010; Liefting, Hoffmann, & Ellers, 2009; Lind & Johansson, 2007) or among individuals of the same population (Hus*by et* al., 2010; Nussey, Wilson, & Brommer, 2007; Porli*er et* al., 2012; Westneat, Hatch, Wetzel, & Ensminger, 2011).

In many lekking or chorusing birds, frogs and insect species, acoustic communication occurs in social settings composed of multiple signallers and receivers (McGregor & Peake, 2000). Males compete to attract conspecific females (Andersson, 1994), which in turn choose among mates based on their signalling displays (reviewed in Andersson & Simmons, 2006). The males' signal traits are sexually selected (Byers, Hebets, & Podos, 2010) and an increased levels of male-male competition causes sexual selection (Souroukis & Cade, 1993). These highly competitive social environments pose distinct challenges to effective communication, since males must ensure their signals stand out from those of their rivals (Bretman, Westmancoat, Gage, & Chapman, 2013; Höbel, 2015; Reichert & Gerhardt, 2012). Rapid fluctuations of social context might favour behavioural plasticity: males must balance the costs of producing effective signals with the need to maintain their status in changing levels of signalling competition (Reichert & Gerhardt, 2012, 2012). Indeed, the production of these conspicuous mate attraction signals might lead to a double cost for signallers in terms of energetic

demand and predators - parasitoids attraction (Zuk & Kolluru, 1998). Clear examples are the predatory behaviours of bats on calling frogs, crickets and katydids (Jones, Page, Hartbauer, & Siemers, 2011) and parasitism of acoustically orienting flies on calling male crickets and katydids (Cade, 1975; Lehmann & Heller, 1998). Extrinsic factors liked to the surrounding communication environment itself (i.e., density and number of signalling rivals, population mobility and operational sex ratio) can influence the magnitude of phenotypic plasticity (Bertram, Harrison, Thomson, & Fitzsimmons, 2013; Bretman, Gage, & Chapman, 2011) and the individual's signalling tactics (Humfeld, 2013). The socially mediated plasticity allows males to deal with high levels of acoustic competition, keep up their signalling efforts and increase investment when it is expected to maximize mating success (Greenfield, 1994; Höbel, 2015; Klump & Gerhardt, 1992; Kuczynski, Gering, & Getty, 2016; Reichert, 2011). Several studies highlighted how males adjust their signals depending on variation in the surrounding social environment, such as presence and proximity of conspecific rivals, as well as competition level (Freeberg & Harvey, 2008; Greenfield, 2005; Price, 2006; Reichert, 2011; Reichert & Gerhardt, 2012). In Orthoptera, males can vary certain call features in response to vocal activity, density and proximity of signalling rival males (Hedrick, 1986; Latimer, 1981; Römer & Bailey, 1986). For instance, during competition, heavier males of *Poecilimon ampliatus* can modify their signal's duration and duty cycle depending on the proximity of rivals (Anichini, Frommolt, & Lehmann, 2018).

There is also a range of intrinsic factors that could alter selection for plasticity (Bretman, Gage, & Chapman, 2011). Body size affects the intensity and frequency of the acoustic signal, and large males generally emit louder songs with lower fundamental frequency (Bailey & Thiele, 1983; Latimer & Schatral, 1986). In crickets, signal duration and duty cycle are strongly positively correlated with body condition (Gray, 1997; Hack, 1997; Scheuber, Jacot, & Brinkhof, 2003; Shackleton, Jennions, & Hunt, 2005; Souroukis & Cade, 1993). Males in poor body condition can therefore only put lower amount of effort into acoustic signalling (Holzer, Jacot, & Brinkhof, 2003; Ritchie, Sunter, & Hockham, 1998; Simmo*ns et* al., 1992; W. E. Wagner & Hoback, 1999), since they might incur higher costs of responding (Bretman, Gage, & Chapman, 2011). Evidences in this regard were also found in light males of *P. ampliatus*, which signalled with shorter verses at reduced duty cycle when competing against heavier males (Anichini, Frommolt, & Lehmann, 2018). In species with lek mating systems, females are mostly attracted by signals of high intensity, long duration and high repetition rate (Gerhardt & Huber, 2002; Greenfield, 2002) and usually choose larger or heavier competing males (reviewed in Gwynne, 2001; Lehmann, 2012).

Because of energetic constraints, individuals of different body mass are likely to vary in their plasticity levels, depending on the interplay existing between the social environment and the condition-dependent signalling ability. A good example of inter-individual variation in plasticity, in dynamic social environments, are male choruses, both of insects and frogs, that exhibit high degrees of

variability in density, composition (Gerhardt & Huber, 2002; Parrish & Hamner, 1997), presence and signalling activity of particular individuals in the chorus (Friedl & Klump, 2005; Murphy, 1994).

In this study, we examined the effects of body condition and the social environment on the expression of plasticity in the acoustic features of bushcricket male mating signals (genus *Poecilimon*). We hypothesized that individual body condition would predict the magnitude of the plastic response to changes in the social environment. The bushcricket *Poecilimon veluchianus veluchianus* (Ramme, 1933) exhibits several features that make it a suitable for studying signalling plasticity. In this species, only males produce acoustic signals, commonly aggregating in choruses to attract conspecific females (Heller & von Helversen, 1993), which in turn evaluate and localize their preferred mate by phonotaxis (Heller, 1992; Heller & von Helversen, 1991, 1993).

We compared the acoustic response of heavy and light focal males in different social contexts created by manipulating both the body mass and number of rivals. Body mass is a reliable predictor of male body condition (Gray, 1997; Hack, 1997; Scheuber, Jacot, & Brinkhof, 2003), therefore light and heavy males can be considered to be in poor and in good body condition, respectively. Since the social environment determines the level of competition (Bretman, Gage, & Chapman, 2011; Greenfield, 2015; Greenfield, Marin-Cudraz, & Party, 2017; Krobath, Römer, & Hartbauer, 2017; Rebar & Rodríguez, 2016) and individuals attend to the signals of conspecific rivals (Greenfield & Shaw, 1983; Latimer & Schatral, 1986; Latimer, 1981), signalling activity of focal males was tested under four levels of competition: 1) one light rival; 2) two light rivals; 3) one heavy rival; and 4) two heavy rivals. The strength of competition was determined by considering both the number of rivals singing and their body mass: one light rival thus represented minimal competition strength, while two heavy rivals represented maximal competition strength.

Assuming that the social context affects the signalling, expression and induces to a plastic response of focal males tested, we predict that focal males are able to alter the magnitude of their acoustic response depending on both the rival body condition and number. Specifically, we expect that focal males will increase their signalling activity when the density of heavy rivals is higher (i.e., the competition pressure is stronger). Furthermore, assuming that plasticity itself is costly (DeWitt, Sih, & Wilson, 1998; Relyea, 2002), we predicted that individuals in poorer condition should be less plastic (i.e. less likely to adjust their signalling behaviour in response to changes in the level of competition).

4.3. Methods

Our study organism, *P. v. veluchianus* (Ramme, 1933), is a medium-sized, nocturnal, herbivorous bushcricket endemic to Central Greece (Eweleit & Reinhold, 2014; Willemse & Heller, 1992). This field study was carried out in June 2016 near the village of Palaiokastro (38°98'00" N, 21°89'90" E), Nomos Fthiotis, Central Greece. Over 2 weeks, we collected 314 adult males, which were initially kept in collective cages (60 x 60 x 60 cm). After 2 days, males were separated and individually placed

in cylindrical plastic jars (13 x 9 cm), covered by a cotton net. They were fed ad libitum with a mixture of fresh leaves and wildflowers. As previous mating history of the collected males was unknown, for the experiments we used only males kept in captivity for 7 to 10 days. This is much longer than the refractory period for sperm replenishment and spermatophore production, which is known to be 2 to 3 days in the field (Heller & Reinhold, 1994) and the laboratory (Reinhold & Helversen, 1997). Afterwards we released the males not used in the experiments into their original habitat, whereas we stored those tested after experiments in 70% ethanol for further morphological examination.

The acoustic signal of *P. v. veluchianus* males consists of repeated verses with pauses of several seconds in between (Heller & Reinhold, 1994; Heller & von Helversen, 1993; Lehmann & Heller, 1998), as shown in Fig. 4-1a. Verses are composed of trains of a variable number of units called syllables (Fig. 4-1b), generated by a single down stroke movement of the forewings (Heller, 1988).

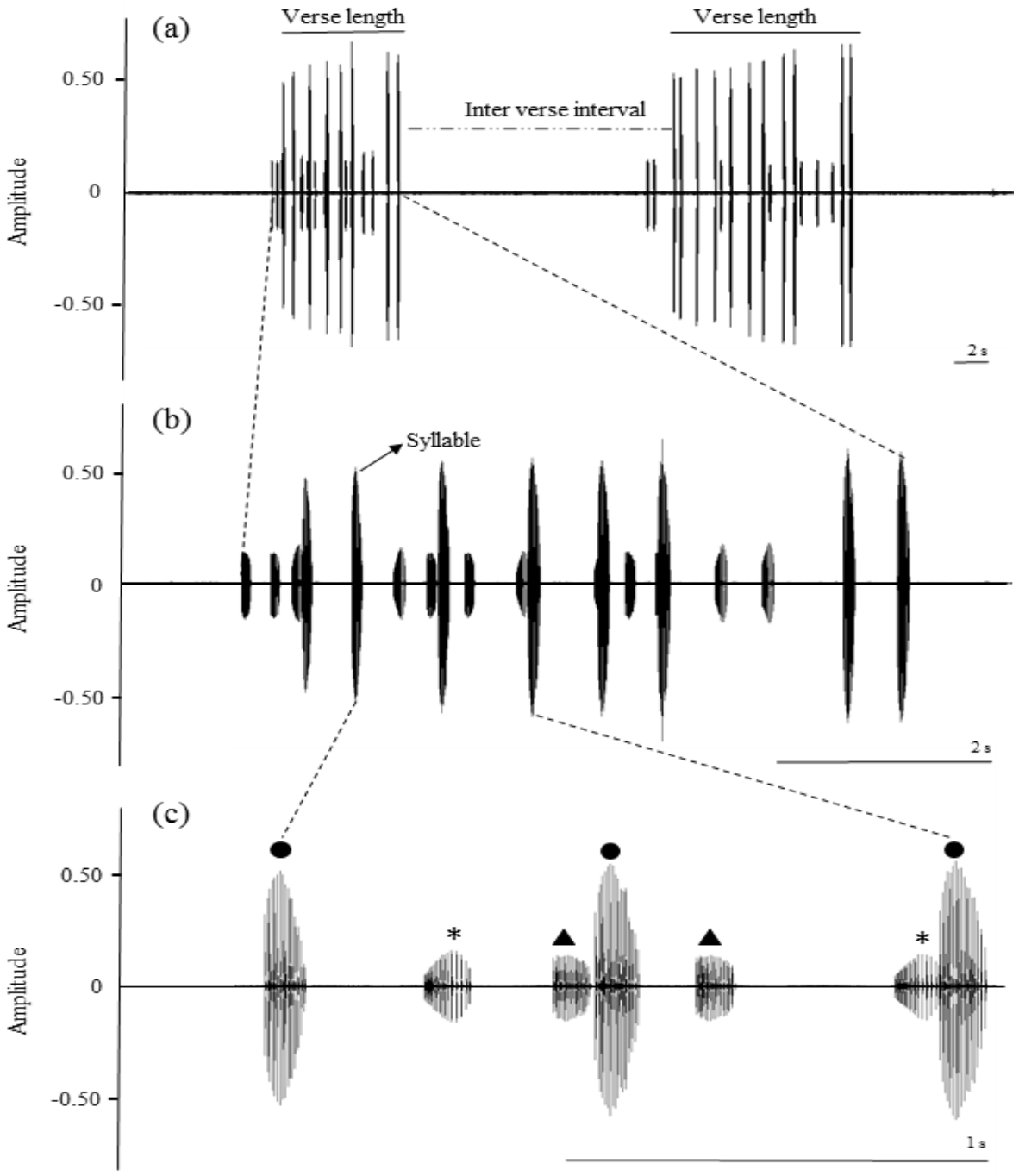

Figure 4-1. Acoustic signal of *P. v. veluchianus* males. Oscillograms showing (a) the length of 2 verses, separated by a silent interval; (b) enlarged section of the first verse composed of 8 syllables; (c) a snippet of 3 syllables of the focal male, marked by solid circles. Two syllables of each of the two rivals signalling at the same time are indicated by different symbols (asterisks and triangles).

4.3.1 Experimental design

We tested signalling performance of a focal male facing two rivals in an enclosure (2 x 2 x 1 m), in order to keep the predators out and prevent acoustic interferences with other males from the local population. The "signalling trio" was placed at the centre of the arena, with the focal male at the vertex of an equilateral triangle, so that all the males were spaced equidistantly by 20 cm (Fig. 4-2). To keep the distance constant and prevent the males from moving, they were recorded in their individual cages. In line with the experiments performed on a related species, *P. ampliatus*, we chosen an inter-male distance of 20 cm to stimulate the males to compete, without compromising the quality of the signal recordings.

We randomly selected 64 males from the chosen *P. v. veluchianus* population and used a digital scale (KERN EMB 200-3 scale; accuracy ± 1mg) to measure their body mass (mean ± SD = 643.2 ± 84.0 mg). From the obtained measurements, we determined the first and third interquartile thresholds for male body mass in this population. Accordingly, all individuals used in this study that weighed less than the lower threshold (594 mg) were classified as light males, while those that weighed more than the upper threshold (690 mg) were classified as heavy males (Table S4-1). Body mass difference of 100 mg was considered the minimum value to significantly separate the two body mass classes, as previously employed in experiments with the congeneric species *P. zimmeri* (Lehmann & Lehmann, 2008) and *P. ampliatus* (Anichini, Frommolt, & Lehmann, 2018).

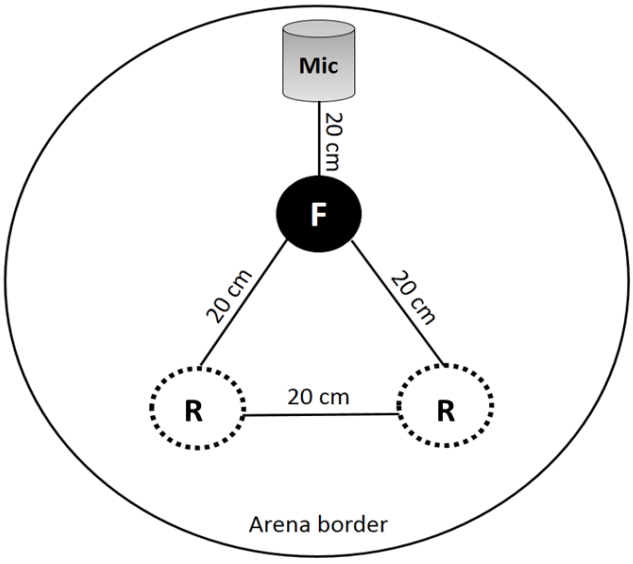

Figure 4-2. Schematic figure, representing the experimental setup in the arena. F= focal male, R = rivals, Mic = microphone.

Every night a set of two light and two heavy males were selected as focal males. Each focal male's signalling activity was tested against two rivals chosen from (a) the same and (b) different body mass classes. Experiments were performed in random order. From this point on rivals' body mass,

either light or heavy, will be referred to as “rival type” in this study. Altogether 16 rivals (4 for each focal male) were used per night, each in a single test. Thus, every signing trio was a unique combination of focal and rival males and the same animals were not used in the following nights.

Using geographical coordinates, we obtained average air temperature in the study area from “Meteoblue” (https://www.meteoblue.com), to include it as control covariate in all statistical analyses (see “Statistical analysis”).

4.3.2 Body mass analysis

In our study, a total of 30 males of *P. v. veluchianus* were selected as focal individuals and their acoustic signals were recorded (Table S4-1). To compose the “singing trio” experimental setup, 28 males were adopted as heavy and 27 used as light rivals (Table S4-1). Body masses of heavy (mean ± SD = 734.5 ± 43.6 mg, N = 46) and light males mean ± SD = 569.7 ± 42.3 mg, N = 43) differed by an average of 164.8 mg. According to the Welch Two Sample t-test, body mass discrepancy between heavy and light males was significant (t = 18.13, df = 86.89, $P < 0.001$). The greatest body mass difference of 174.0 mg was found between light and heavy males adopted as rivals (Table S4-2, Fig. S4-1). On the contrary, males belonging to the same body mass class (either focal or rival) differed by only about 14.0 mg in their body mass, and this discrepancy was not significant (Table S4-2, Fig. S4-1).

4.3.3 Signal recording

Acoustic signals of the focal males were recorded during the peak period of nightly signalling activity in P. v. veluchianus, between 11:00 pm and 03:00 am (Heller & Reinhold, 1994; Heller & von Helversen, 1993). Males were given 10 min to acclimatize before the recordings. A microphone was placed 20 cm above the opening of the cage containing the focal male. By placing the microphone closer to the focal male than to the two rivals, we were able to identify the signal of the focal male, due to higher amplitude representation of its signal (Fig. 4-1c). Acoustic activity of the males was recorded for 3 min using UltraSound Gate 116-200 (Avisoft Bioacoustics, Berlin, Germany), composed of a single ultrasound microphone (frequency range 10 - 120 kHz), 4 m cable (with NCMX connector) and Avisoft-RECORDER USGH software connected to a laptop. Sounds were recorded at a sampling rate of 250 kHz to cover the entire frequency range. Recordings were stored in PCM format as .wav files. Avisoft-RECORDER USGH software provided a real-time spectrogram display on the laptop, allowing for visual inspection. To eliminate low frequency background noise, digital signals were filtered in Audacity software version 2.2.2 (https://www.audacityteam.org) with a high-pass of 1 kHz. This filter is far below the frequency range of P. v. veluchianus signals, which is 20 to 40 kHz (with a mean value of 23.34 kHz) (Rebrina et al., n.d.).

4.3.4 Signal analysis

Signal features were measured manually from the waveforms in Audacity software version 2.2.2 (https://www.audacityteam.org). In the analysis, we only included the recordings reporting signals of focal males in competition against a consistent number of signalling rivals. Differences in acoustic features, namely the shape and the intensity of syllables, were adopted to identify the number of signalling rivals and distinguish their acoustic output from that of the focal male (Fig. 4-1c). We discarded 33% of audio data from the total recorded, because these files did not meet the criterion adopted in this study of "consistent amount of rival signalling". This criterion states that for at least 90% of the whole file (2.7 min over a total duration of 3 min), the recordings have to include the signal of either only one or two rivals. Thus, we were able to introduce the variables "rival number" in addition to "rival type" in our analysis.

For a random subsample of our focal male recordings, we determined that the inter-verse interval was on average about 10 s long (Fig 4-1a). Accordingly, in this study, two groups of syllables were considered distinct verses if they were separated by a silent interval of at least 10 s.

From the total 3 min. recording duration, we randomly chose a 90 s time frame within each recording. In this time frame, we focused on the temporal signal properties, typically involved in signal adjustment (Anichini, Frommolt, & Lehmann, 2018; Greenfield, 2005). Specifically, we examined 4 acoustic features of the focal males' signals: verse duration (Fig. 4-1a), verse number, total number of syllables and duty cycle. Duty cycle was calculated as the ratio of the total time a focal male spent signalling (sum of all verse durations), divided by the total recording time considered (90 s).

4.3.5 Statistical analysis

Data was analysed using R version 3.4.4 (R Development Core Team, 2018) and R-Studio version 1.1.442 (R Studio Team, 2018). In *P. v. veluchianus* signal features covary (Rebrina *et al*., in prep.) and thus do not represent independent data for our analyses. Therefore, we performed a principal component analysis with the function "PCA" from the package "FactoMineR" (Husson, Josse, Le, Mazet, & Husson, 2018), to obtain uncorrelated measures of the focal males' signal features. We used PC-scores with eigenvalues greater than 1.0 (Table S4-3, Fig. S4-2) as response variables in linear mixed-effects models (LMM), fitted with restricted maximum likelihood estimation (REML) with the function "lmer" from the package "lme4" (Bates, Mächler, Bolker, & Walker, 2015). From the percentage of contribution of each signal features measured, we determined the importance of their variance in the whole PC1 (Table S4-4).

To test the acoustic response of light and heavy focal males in relation to different combinations of rival type and number, we included a three-way interaction between the predictor variables as a fixed term. Each variable had two levels: focal male body mass (either "heavy" or "light"), rival type (either "heavy" or "light") and rival number (either "one" or "two"). The standardized value of the

average temperature was included as a fixed factor in the model. According with Bates, Mächler, Bolker, and Walker (2015) and Pinheiro and Bates (2000), likelihood ratio test (LRT) was carried out with the function "anova", from the package 'stat', to compare different models with respect to the random effect. From the *P* value obtained, we determined whether changing the random terms influenced the predictive ability of the model (Bates, Mächler, Bolker, & Walker, 2015; Pinheiro & Bates, 2000). Following the Akaike Information criterion (AIC; Akaike, 1998), we used the AIC values resulting from the comparison of the models as further confirmation of the model's fit (Table S4-5). The first model (1) presented only the fixed term, as interaction between "focal male body mass * rival type * rival number". The second model (2) included the random slopes for "male ID F" specified, so that both the effect of variation in focal males on the intercept (random intercept) and the effect of variation in rival number (random slope) are included, and the variances for the two levels of rival number are allowed to be different. The third model (3) included the effect of variation of rival type in the random slope specified for "male ID F", and the potential heteroscedasticity with respect to the two levels rival type is modelled. The fourth model (4) presented the full random slopes term, including both rival number and rival type, specified for "male ID F". While the random slope including the variation in rival type significantly increased the fit of the model and decreased the AIC value, this was not the case with the random slope including rival number, both in models (2) and (4) (Table S4-5). Consequently, for the subsequent analyses, we adopted the best model (3), which included only the slope in rival type as random factor. This model presented the best fit and the lowest AIC value (Table S4-5). We calculated *P* values for fixed effects by performing a type III ANOVA on the model, with the function "anova" from the package "lmerTest" (Kuznetsova, Brockhoff, & Christensen, 2015). The evaluation degrees of freedom are based on the Satterthwaite approximation. In case of a significant interaction, a post-hoc analysis of the contrasts was performed, with the function "lsmeans" from the package "lmerTest" (Kuznetsova, Brockhoff, & Christensen, 2015). We visually checked for the validity of the assumptions of linearity, homoscedasticity and normality, using diagnostic plots, showing the standard fitted vs. residual and quantile-quantile plots (Bates, Mächler, Bolker, & Walker, 2015).

4.4. Results

4.4.1 Signal features of focal males

To obtain uncorrelated measurements of the males' signalling responses, based on the four signal features considered, we performed a principal component analysis. Only PC1 has been included in the analysis, since it presented an eigenvalue larger than one and explained 77.64% of the total variance in the measured signal features (Table S4-3, Fig. S4-2). We therefore used the PC1's scores as the dependent variable to analyse the focal male's signal output.

The signal features, verse duration, duty cycle and total syllable number, presented high and similar values in both loadings and percentage contributions to the total variance in PC1 (Table S4-3, S4-4). In contrast, the verse number presented the lowest loading (Table S4-3) and its percentage of the variance was not significantly high to explain the total variance in PC1 (Table S4-4).

4.4.2 Signalling response of focal males

Focal males varied their temporal signal features depending on their body mass and the surrounding social environment. The output of the LMM on PC1 showed a significant three-way interaction between focal male body mass, rival type and rival number (Table 4-1). As detailed below, only heavy individuals adjusted their signalling response (Fig. 4-3, left panel).

Table 4-1. Acoustic features variation of *P. v. veluchianus* males.

Factor	Numerator *df*	Denominator *df*	*F*	Pr (> *F*)
Body mass	1	22.56	0.84	0.370
Rival type	1	17.58	8.81	**0.008**
Rival number	1	28.39	0.19	0.666
Body mass * rival type	1	17.53	1.17	0.294
Body mass * rival number	1	28.48	0.17	0.681
Rival type * rival number	1	34.93	10.16	**0.003**
Body mass * rival type * rival number	1	32.38	8.74	**0.006**

Signal features of focal males were tested for the influence of focal male body mass (heavy, light body mass), rival type (heavy, light body mass), rival number (one or two) and their interaction. Significant probabilities of the model performed in PC1 with Satterthwaite approximation and control of the effect of temperature are in bold. Number of observations = 60, number of males = 30.

PC1 score for heavy focal males was significantly lower when facing two heavy rivals than when facing only one (df = 24.4, t = - 3.19, $Pr(>|t|)$ = 0.004). At an increased density of stronger rivals, heavy focal males were therefore signalling with shorter verses, lower duty cycles and a reduced number of syllables (Fig. 4-3, left panel). However, when facing light rivals the opposite result was found: heavy males' signalling response was stronger against two light rivals than against only one (df = 17.0, t = - 2.31, $Pr(>|t|)$ = 0.034). Heavy focal males significantly increased signal and verse duration and overall signalling effort when the number of light rivals increased (Fig. 4-3, left panel).

Although heavy males did not significantly modify their signal features depending on rival type when facing a single competitor (df = 22.1, t = 0.98, $Pr(>|t|)$ < 0.339), they did vary their signalling response depending on the rival type when facing two competitors. Heavy males' response was stronger in competition with two light than with two heavy rivals (df = 32.8, t = 4.59, $Pr(>|t|)$ <

0.001; Fig. 4-3, left panel dashed lines). A significant increase in the signalling response was also found when heavy males were competing against two light rivals in comparison to competing against one heavy rival (*df* = 17.7, t = 2.21, *Pr (>|t|)* = 0.040). There was no statistical significance in the increment of signalling response between heavy males contesting against one light rival in comparison to contesting against two heavy rivals (*df* = 27.1, t = 2.87, *Pr (>|t|)* = 0.101).

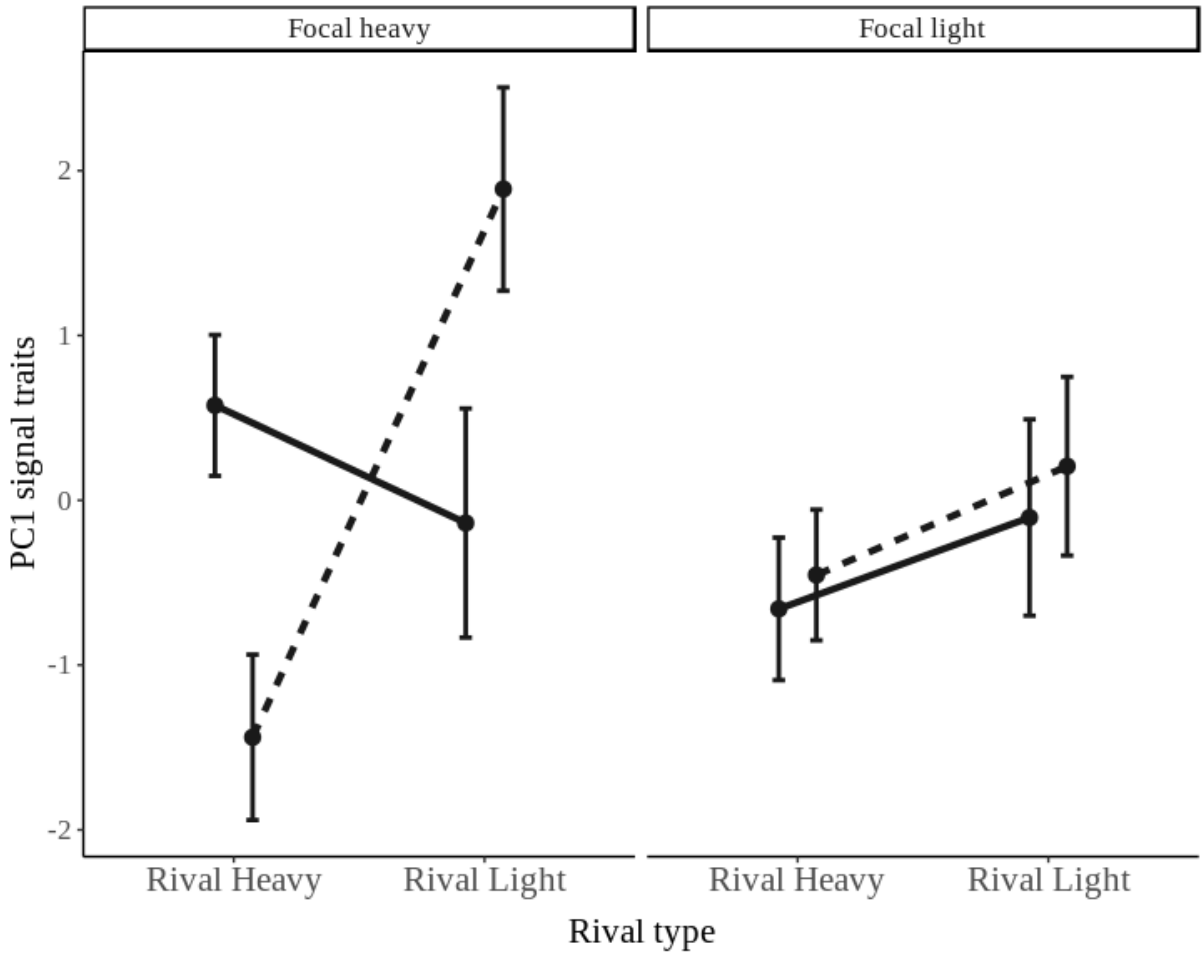

Figure 4-3. Effect of body mass, rival number and rival type on the focal males' signal features in PC1, for heavy focal males (left) and light focal males (right). Points and error bars show fitted values and 95% confidence interval, respectively. Solid line represents one rival, dashed line represents two rivals.

PC1 scores for light focal males did not vary significantly, indicating that light focal males did not vary their signal features with either rival type or rival number (Fig. 4-3, right panel).

Comparing light and heavy focal males tested under the same competition pressure, only marginally significant differences were found. Heavy males signalled with a stronger effort than light individuals, both when they were competing against two light rivals (*df* = 23.8, t = 2.04, *Pr (>|t|)* = 0.052) and against one heavy rival (*df* = 25.8, t = 2.51, *Pr (>|t|)* = 0.057). Regarding differences in

response to two heavy rival males, the acoustic effort of light and heavy focal males did not significantly differ in that constellation (*df* = 23.1, t = 1.54, *Pr (>|t|)* = 0.137). In contrast, we found that light focal males in competition with two light rivals responded more strongly than heavy focal males in competition with two heavy rivals (*df* = 48.6, t = 2.23, *Pr (>|t|)* = 0.030).

4.5. Discussion

In our experiments, *P. v. veluchianus* males adjusted their temporal signal features depending both on their body condition and the social environment. These findings underline the importance of examining the interaction between intrinsic and extrinsic factors to understand inter-individual variation in signalling plasticity. Both the type and number of rivals present in the environment markedly influenced the variation in the mean plasticity level at two scales: between individuals of similar and different body mass classes. We discuss these results in more detail below.

Differences in signalling between individuals of similar body mass.

Only heavy focal males varied their signal performance depending on both type and number of rivals. When facing two heavy rivals, heavy males showed the lowest signalling effort, while they showed a very high signalling effort when competing against two light rivals. In contrast, when facing only one rival, heavy focal males' acoustic activity was not affected by the rival type. If the number of light rivals increased from one to two, heavy focal males showed an increment of their signalling activity. In contrast, if the number of heavy rivals increased from one to two, heavy focal males substantially decreased their acoustic activity. These findings suggest that *P. v. veluchianus* males perceive the competition level in the surrounding environment, and accordingly adjust their signal production depending on their own body condition. In a high-density chorus, males are frequently surrounded by many conspecifics, functioning both as signallers and receivers (Reichert, 2011). Males may gain information about the size, vigour and fighting ability of rivals from their acoustic signal characteristics (Greenfield & Shaw, 1983; Latimer & Schatral, 1986; Latimer, 1981), and the increased level of competition generally leads to an increment of male signalling performance (Anichini, Frommolt, & Lehmann, 2018; Reichert & Gerhardt, 2012). However, our new results only partially correspond to the finding that increased levels of competition generally lead to an increment in male signalling performance. Under the strongest competition heavy males drastically decreased their signalling effort instead of increasing it as would be expected. It is likely that these individuals perceived the presence of the second rival in good body condition as an increment in the competition pressure. This, in turn, led to increased expression of their plastic behaviour, depending on the rival type they were facing: an increase or a decrease in acoustic effort when competing against two light or two heavy rivals, respectively.

As far as we know, no previous studies dealing with plasticity in signalling behaviour have considered both body condition and number of rivals as contributing factors. The number of signalling rivals alone has been considered in several studies, mainly to investigate call timing and selective attention in chorusing insects and anurans. These results revealed that males pay attention to a small but not necessarily singular number of nearby individuals (Brush & Narins, 1989; Greenfield & Rand, 2000; Greenfield & Snedden, 2003; Snedden, Greenfield, & Jang, 1998). Reichert (2011) further demonstrated that treefrog males are affected by the number of simulated rivals on the one hand and the type of their calls on the other hand. Further field experiments in frogs showed that variation in the number and density of attractive signalling neighbours directly affect a male's relative attractiveness and his future mating strategies (Davies & Halliday, 1978; Wagner, 1989). Our findings show that competing males pay attention not only to the number but also to the type (in terms of body mass) of rivals singing in the neighbourhood. Since body quality is honestly advertised through specific features of acoustic signals, especially under competition (Anichini, Frommolt, & Lehmann, 2018; Parker, 1974; Reichert, 2014), focal males might be able to assess the body condition of the rivals by their acoustic signals.

Nevertheless, these results pose an interesting question: why did heavy focal males change their signalling effort only when competing against two, but not against a single rival?

In general, males may not benefit from attempting to compete against many rivals in good body condition, so they may stop signalling as soon as the signalling effort ceases to be advantageous. It might be that the higher competition pressure, due to the introduction of the second heavy rival, resulted in the focal male's signals being too ineffective under that level of competition to be worth continuing to signal. Density, phenotypic characteristics and demographic composition of individuals interacting within a group may influence a male's fitness in many species (Farr, 1980; Jirotkul, 2000; Oh & Badyaev, 2010) including bushcrickets (Lehmann, 2007), and lead to alternative behavioural strategies. To a male signalling in an intense competitive environment might be more advantageous to remain in silence, move away or perform satellite behaviour. In crickets, a relation between silent behaviour at higher densities is found (Hissman, 1990; Simmons, 1986) and the frequency of satellite behaviour increases with male population density (Cade & Cade, 1992). Furthermore a male might adopt spacing behaviour**,** choosing to move in other spots where their calls can be more effective (Arak & Eiriksson, 1992; Farris, Forrest, & Hoy, 1997; Forrest & Green, 1991). Being closer to the rivals of inferior condition, males might increase their relative attractiveness. Our results showed that heavy males put more effort in their signalling when competing against two light rivals than when competing against two heavy rivals. To date, however, few empirical studies have attempted to test whether competing males indeed benefit from associating with inferior rivals (Bateson & Healy, 2005; Oh & Badyaev, 2010). Callander, Hayes, Jennions, and Backwell, (2012) showed that during competition for mates, larger fiddler crab males are more likely to attract females if they display close

to smaller rivals. This study confirmed that the body condition of neighbouring rivals can affect a male's attractiveness and, hence, it's mating success.

Differences in signalling between individuals of different body mass.

In our study, heavy focal males exhibited high plasticity in signalling behaviour, significantly varying their acoustic effort depending on the social environment. Conversely, light focal males did not show any plastic signal behaviour. Their signal features were only slightly influenced by the variation in both number and type of signalling rivals. Our results suggest that heavy males were more responsive to changes in the social environment than light males. These findings partly extend what was found in a previous study that investigated the signalling activity of light P. ampliatus males in competition against heavy rivals, placed at different distances to simulate different levels of contest (Anichini, Frommolt, & Lehmann, 2018). Anichini, Frommolt, and Lehmann (2018) showed that light males did not exhibit any plastic response depending on the strength of the competition: the acoustic production of light males did not change between isolation and the strongest competition condition (having the heavy rival placed in front).

However, these results bring up an interesting question: why do lighter males, supposedly in poorer body condition and thus less competitive than heavy males, not reduce their signalling effort or withdraw from the highly competitive contest? Given that the chances of being chosen by a female are generally low for a male in poor body condition, especially when competing against more attractive rivals, it is possible that these males choose to keep on displaying near superior rivals in order to increase their likelihood of mating with the females attracted by better-displaying males (i.e. "hotshot" models of lek formation, Beehler & Foster, 1988). Alternatively, the trade-off between being an efficient signaller and coping with the costs of behavioural adjustments might provide a further viable explanation (DeWitt, Sih, & Wilson, 1998; Relyea, 2002). Acoustic signalling in animals is under strong sexual and natural selection (Bailey, 2003; Bradbury & Vehrencamp, 1998; Gerhardt & Huber, 2002; Greenfield, 2002). Developing and maintaining structures necessary for sound production and perception entails high costs, which could depend on male body condition (Del Castillo & Gwynne, 2007). Namely, if *P. v. veluchianus* males possessed fine perceptual abilities to elaborate acoustic cues (rather than just an on/off mechanism regardless of the external conditions), one would expect the males in good body condition to be more efficient in facing higher "*maintenance costs"* of the sensory system than the males in poor body condition (DeWitt, Sih, & Wilson, 1998). This could lead to a lower ability of the latter to assess the social environment, which may account for the lack of signalling behaviour plasticity in light focal males. However, further studies are needed to validate this inference.

4.6. Conclusions

Manipulating both rival body condition and the number of rivals in a more complex experimental setup, which goes beyond the dyadic interactions mostly experimented so far, allowed us to test the magnitude of plasticity in signalling behaviour in P. v. veluchianus males. Our findings show the decisive role played by the social context, in determining the acoustic responses of competing males presenting either similar or different body condition. The variation in plastic signalling responses found in heavy males, and the lack of plasticity in light males, is intriguing and opens further questions. However, important is to underline that in this study we used caged P. v. veluchianus males. Thus, acoustic signalling is the only component considered in this study between the displays adopted for mate attraction. To discover how males alter multiple aspects of their courtship behaviour under different levels of competition, it is crucial to empirically investigate the link between condition dependent signalling and alternative behavioural strategies.

4.7. Acknowledgement

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4.8. Supplementary Information

Table S 4-1. Descriptive statistics of *P. v. veluchianus* male body mass.

Body mass code	*N*	Mean	SD	SE	CI
Focal H	16	731.13	41.81	10.45	22.28
Focal L	14	585.07	34.52	9.22	19.93
Rival H	30	736.28	45.07	8.23	16.83
Rival L	29	562.31	44.02	8.17	16.74

Body masses of focal and rival males are included. Legend: Focal H = heavy focal males, Focal L = light focal males, Rival H = heavy competitors, Rival L = light competitors, *N* = number of individuals

considered, Mean = Average value, SD = Standard deviation, SE = Standard error of the mean, CI = Confidence interval (95%).

Table S 4-2. Multiple comparison of *P. v. veluchianus* male body mass.

Body mass comparisons	**diff**	**lwr**	**upr**	***P* adj**
Focal L – Focal H	-146.05	-186.99	-105.12	**0.000**
Rival H – Focal H	5.16	-29.47	39.79	0.980
Rival L – Focal H	-168.81	-203.65	-133.98	**0.000**
Rival H – Focal L	151.212	115.00	187.42	**0.000**
Rival L – Focal L	-22.76	-59.17	13.64	0.363
Rival L – Rival H	-173.97	-203.10	-144.84	**0.000**

Tukey post hoc test output of the comparative analysis at 95% family-wise confidence level. Legend: diff = difference in the observed means, lwr = lower interval range, upr = upper interval range, *P* adj = *P* value after adjustment for the multiple comparisons.

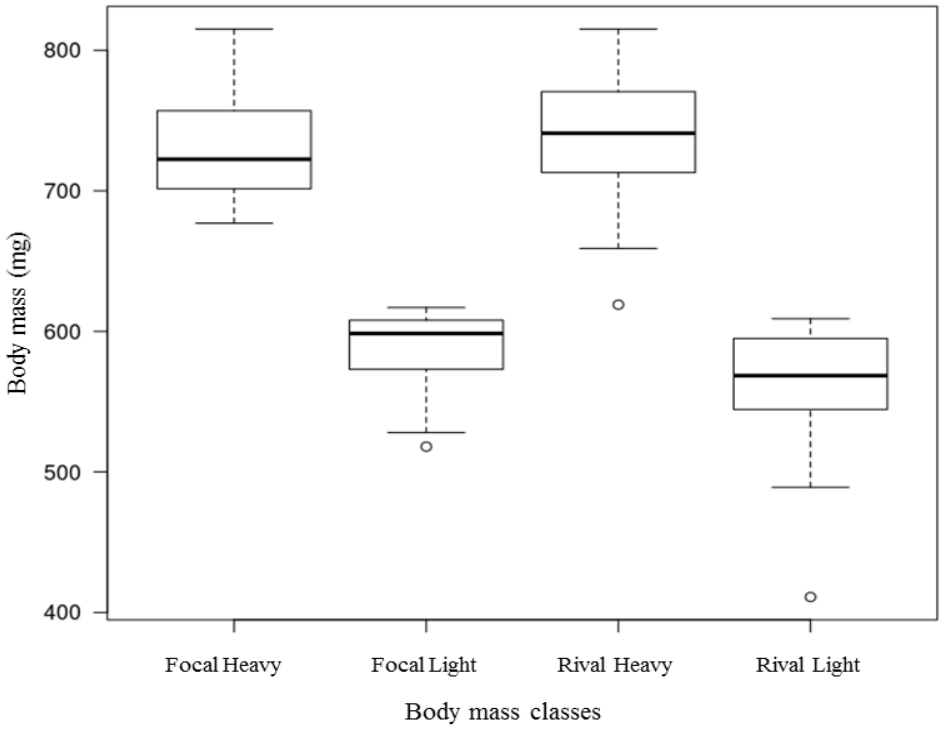

Figure S 4-1. Box plot illustrating the comparative analysis of *P. v. veluchianus* body mass. X-axis = male body mass classes, y-axis = body mass in milligrams (mg).

Table S 4-3. Variable loadings for each signal features of *P. v. veluchianus* males.

Signal features	**PC1**	**PC2**
Verse number	0.12	**0.94**
Total syllable number	**0.89**	0.32
Verse duration	**0.79**	-0.49
Duty cycle	**0.86**	0.39
Eigenvalue	3.11	0.72
Variance explained	77.64	17.89

Unrotated coordinates of variables loadings in PC1 and PC2, which made an important contribution to the components are indicated in bold. Only PC1 was considered in the analysis.

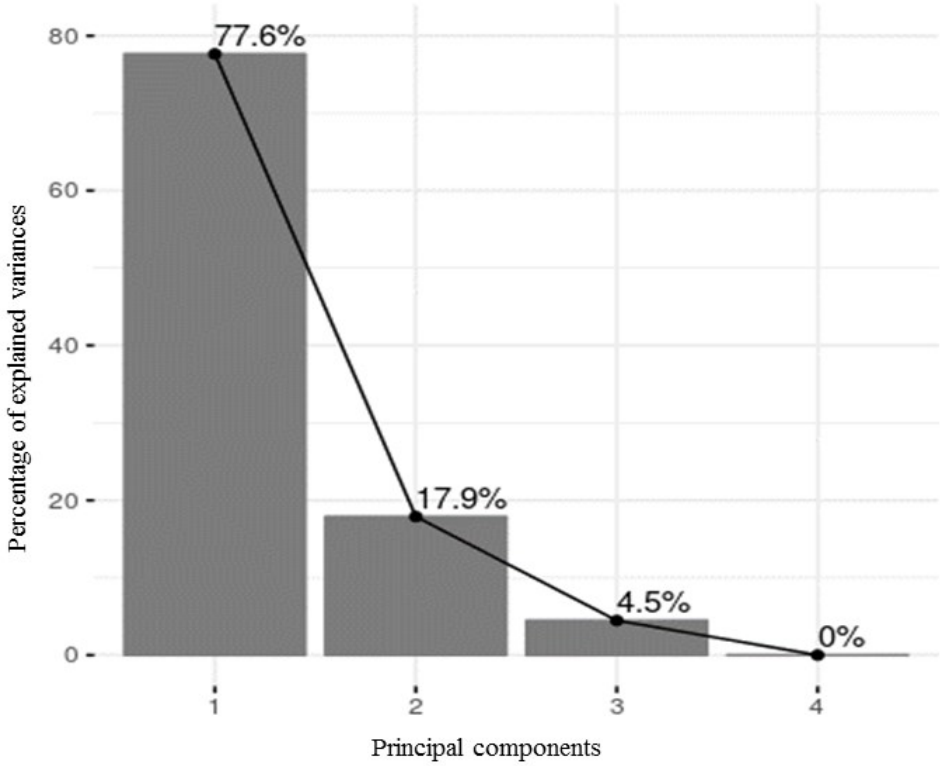

Figure S 4-2. Scree Plot of eigenvalues ranked from the largest to the smallest. The number of components (PCs) is reported on the x-axis and the percentage of the variance explained by each PC is shown on the y-axis.

Table S 4-4. Percentage of contribution for each signal features of *P. v. veluchianus* males.

Signal features	**% contribution in PC1**
Verse number	11.90
Total syllable number	**27.39**
Verse duration	**30.37**
Duty cycle	**30.33**

Percentage of contribution of the male signal features to the principal component PC1. In bold are the percentages that add an important contribution to the total variance of PC1.

Table S 4-5. Random term model's comparison adopting PC1 scores as answering variable.

Model	Fixed term	Random term	*Df*	AIC	BIC	logLik	deviance	χ^2	*Df* (χ^2)	*Pr* (>χ^2)
1	lm (PC1 ~ rival type * rival number * Body mass focal male)		10	232.46	253.40	-106.23	212.46			
2	lmer (PC1 ~ rival type * rival number * Body mass focal male)	(1 + rival number | male ID F)	13	228.74	255.96	-101.38	202.74	9.73	3	0.021
3	lmer (PC1 ~ rival type * rival number * Body mass focal male)	(1 + rival type | male ID F)	13	**224.76**	251.99	-99.38	198.76	3.98	0	**< 0.001**
4	lmer (PC1 ~ rival type * rival number * Body mass focal male	(1 + rival type + rival number | male ID F)	16	228.76	262.27	-98.38	196.76	2.00	3	0.573

Comparison between the models fitted using restricted maximum likelihood (REML) estimation. AIC: Akaike information criterion; BIC: Bayesian information criterion. Significant *P* values and lowest AIC values are written in bold. Model's random effects definition: restricted model presenting only the fixed term 1, random slope model with rival number 2 = (1 + rival number | male ID F), random slope model with rival type 3 = (1 + rival type | male ID F), full random slope model, including rival type and rival number 4 = (1 + rival type + rival number | male ID F).

Chapter 5. Synthesis

5.1. Summary

In Darwin's second book on sexual selection theory, it was described how the male secondary sexual characters evolved, because females generally preferred to mate with individuals that possess those features. On the one hand, these traits are costly and conspicuous, and therefore attract not only mates but also rivals and predators, but on the other hand, they display fitness and give an individual an advantage over its rivals in courtship and aggressive interactions. In competition for mates, males tend to adjust their mate attraction signals according to internal factors, such as body condition and energetic state, and external factors, such as predation risk and competitor density. Thus, behavioural plasticity in sexual selected traits can have an important impact on the fitness outcome. In many species of frogs and insects, the male acoustic communication signals developed under sexual selection. Acoustic signals can be considered as honest cues and their structure might change depending on the physical characteristics of the sound generator organs, male quality and the surrounding social environment.

Because signalling males must balance their own energetic status with the competing demands of attracting mates, repelling rivals and avoiding predators, it remains a crucial issue to investigate how an individual honestly manifests his secondary sexual features and under which conditions the adjustments of these traits is advantageous.

Few organisms are as suitable for such interdisciplinary endeavours as bushcrickets. The morphological and acoustic basis of their mating behaviour is well understood. In many tettigoniid species, only the males signal using a tegminal stridulation structure, while females approach them phonotactically. Sound production is a fundamental behaviour to attract conspecific partners and to compete against rivals, and it depends on internal and external factors related to the signalling male. Larger individuals produce sound at lower frequencies and can signal louder and for longer, while they can also modify the timing and the duration of their signals depending on the neighbouring rivals. Acoustic signals are energetically costly and reflect the quality of the sender: males in poorer condition produce less attractive signals compared to males in better condition. Females prefer to mate with males showing determinate features, transmitted through their sound. During mating, males transfer a spermatophore to the females, which generally choose the heavier individual between two competitors. The females' choice here is adaptive, since heavier males normally produce bigger spermatophore. By selecting the heavier individual through his acoustic cues, females can increase the direct benefit, receiving larger nuptial gifts.

The aims of this thesis are twofold: to understand the role that sexual selection plays in (1) the evolution of morphologies implied in signal producing, and in (2) the production and plasticity of males' acoustic signals adopted in competition for mates.

To accomplish these goals, two congeneric bushcrickets species of the genus *Poecilimon (Insecta: Orthoptera: Tettigoniidae)*, *Poecilimon ampliatus* and *Poecilimon v. veluchianus*, served as a

model. In this thesis, both morphological and audio data of *P. ampliatus* are presented, while, from *P. v. veluchianus,* only audio data have been shown. In both species, the multilevel acoustic signal, produced only by males, varies in the temporal pattern on three timescales: (i) pulses, each produced by a single closing movement of the forewings, (ii), syllables of pulses which can be relatively short and regularly interspaced by pauses and (iii), verses consisting of long trains of syllables which are then also separated by pauses of several seconds in between (*P. ampliatus*: **Fig. 3-1**; *Poecilimon v. veluchianus*: **Fig. 4-1**). In this thesis, the acoustic variables, duration of verses, number of verses, number of syllables and duty cycle have been recorded and analysed under both laboratory and field conditions (**Fig. S1-2**; **S1-3**).

The following section summarizes the main findings of the thesis's core (**Chapters 2 - 4**) alongside the three main research questions, which are introduced in more detail in **Chapter 1**.

Research Question I: Is the morphology of the signalling apparatus an honest cue of male quality, and how are these characteristics selected?

The focus of **Chapter 2** was to investigate the morphology and scaling relationships of the sound production organs in *P. ampliatus* males. To obtain the morphological data, the left-wing area, the total length of the stridulatory file, and the number, length and density of the teeth present on the middle part of the stridulatory file were assessed (**Fig. 2-1**; **Table S2-1**). As reference values of body mass and body size, respectively, the body weight and the length of males' hind femur were measured. Allometric relations between body dimensions and sound generator organs were analysed.

The results reveal seven significant relationships between the morphological traits measured. Firstly, the trait of wing area increases with body mass and body size, and secondly the teeth and stridulatory file length increase as wing area increases. By contrast, both the number and density of stridulatory teeth decrease when the body mass, wing area and stridulatory file length increase. The majority of the scaling relations between factors were either proportional (= isometric) or disproportional (= hypoallometric), suggesting that either a stabilizing or a directional selection pressure on lighter males is likely to be occurring in this species. Only wing area showed a positive hyperallometric scaling, and this positive selection reflects how larger males possess disproportionally larger wings than smaller males. Lastly, heavier and larger males owned longer stridulatory files, with longer and more spaced stridulatory teeth. Thus, stridulatory teeth show a size constraint: with increasing the length, teeth require more space. Therefore, heavier males have larger but fewer stridulatory teeth, suggesting that the body mass is a key factor in determining the stridulatory teeth number and density. These findings reveal that selection plays a role in the allometric relationships between morphological traits of sound producing organs and they imply that better conditioned individuals may have more resources for developing and maintaining exaggeratedly expressed traits than lower conditioned males.

After this initial study concerning the size of acoustic generating structures and their role in reflecting the body mass of the signalling male and, hence, his quality, **Chapter 3** investigated whether acoustic signals produced under competition are a reliable cue for male body condition.

Research Question II: Is the acoustic production an honest predictor of male body condition when males compete against a rival placed at different distances?

To gain insights into how signals from males of different body conditions vary and which acoustic traits might transmit information about their quality, the temporal acoustic features produced by males of *P. ampliatus* were investigated. Two classes of male body mass, light and heavy, were defined. Pairs of light and heavy signalling individuals, of the same age, were recorded both in isolation and in competition. Competing males were placed at different distances to test them under different levels of competition (**Fig. S1-2**): the shortest inter-male distance (10 cm) was the maximum level of competition. The duration of verses and pauses, the number of verses and the total time that males spent signalling (duty cycle) were automatically extracted from the recordings (**Fig. S3-1**).

The findings show no significant discrepancy in the signal features produced by light and heavy males, either in isolation or at the lowest level of competition (inter-male distance: 240 cm). In contrast, heavier males signalled with longer verses and higher duty cycles than the light rivals under high (inter-male distance: 10 cm) and medium levels (inter-male distance: 60 cm) of acoustic contest, respectively, with the rival at the closest and intermediate distances. During the most escalated competition level, only heavier males increased their signal investment up to the maximum, signalling with longer verses and higher duty cycles in comparison to all other competition levels. This result highlights that only heavy males adjusted their acoustic traits accordingly to competition levels, and that an inter-individual variation in males' plastic acoustic behaviour exists. The light males did not adjust their acoustic activity depending on the competition strength and neither did they overcome the signals of heavier rivals with whom they were competing.

In this study, the two factors investigated, male body mass and rival proximity, interact together. Their effect plays a crucial role in causing the variation of males' acoustic traits in signalling activity under competition. Thus, individuals of different body mass, likely because of energetic constraints and costs related to signal production, tend to vary in their acoustic response when contesting. The costs imposed by signal production under the strongest competition pressure might prevent light, low-quality males from signalling with higher quality acoustic traits, in contrast to what heavy, better quality males can achieve.

The next step in assessing the role played by body mass and acoustic environment in male signalling plasticity was shown in **Chapter 4**.

Research Question III: Is the signalling plasticity a good predictor of body condition when males compete against rivals of different body mass and number?

In the fourth chapter of this thesis, the study of male signalling behaviour under competition was extended. In a semi-natural chorus, the acoustic production of *P. v. veluchianus* males was recorded (**Fig. S1-3**). Depending on the individual body mass, two classes of focal males, light and heavy, were determined. The variation of the temporal acoustic features produced in males' acoustic response was measured and compared under different social environments. Four different competition levels were created, combining the rival's body mass and number: from the lowest, with only one light rival signalling, to the highest, with two heavy rivals signalling.

We found that only heavy focal males modified their acoustic response, displaying a significant plasticity in their signalling behaviour, depending on both the rival's body mass and number. Under the strongest competition pressure (against two heavy rivals) they signalled with the minimum effort, using shorter verses, reduced number of syllables and the lowest duty cycle, compared with when they were competing against one heavy rival or two light rivals. In competition with light rivals, heavy focal males signalled more when facing two rivals than only one. In contrast, light focal males did not vary their signal traits, which remained almost constant through different competition levels, indicating that light focal males did not vary their signal temporal features depending on either the rival's body mass or number.

In agreement with what was found in *P. ampliatus* (**Chapter 3**), heavier males of *P. v. veluchianus* were also more responsive to changes in the social environment than lighter males. This evidence likely suggests that the heavy males might be more efficient in paying the costs required by plastic responses. In total, all results found in both species (**Chapter 3-4**) suggest that body mass and social context drive the inter-individual variation of the males' ability to adjust their acoustic features, according to different competition levels. In particular, as shown in *P. v. veluchianus*, the contesting males pay attention not only to the number but also to the quality (in terms of body mass) of rivals singing in the neighbourhood, confirming that these two components of the acoustic environment can affect the male's attractiveness and, hence, the male's mating success.

5.2. Main conclusions

The goal of the present dissertation was to extend knowledge on the role that sexual selection has in the signalling behaviour of bushcrickets males, combining evidence from both morphological and empirical investigations. The results presented in this thesis allow the following main conclusions to be drawn.

The size of sound generator organs is an honest predictor of male body condition.

The results of **Chapter 2** show that heavier males of *P. ampliatus* develop larger wings with longer stridulatory files. They also possess larger but fewer, less densely spaced stridulatory teeth than lighter individuals. Thus, male body mass is a reliable indicator of larger sound producing structures in this

species. However, similar findings are also expected in *P. v. veluchianus* (Rebrina *et al.,* in prep), in light of the previously described similarities which this species have with *P. ampliatus*.

The evidence reported in **Chapter 2** agreed with the general trend shown in a previous comparative research study performed in 58 other bushcricket species (Montealegre-Z, 2009). This study draws similar conclusions for *P. ampliatus*, except for the hyperallometric relation found between wing area and body size. This positive selection, acting on the size of wing, might lead to functional consequences for the acoustic parameters in the male's signal. Larger sound-generating organs are generally associated with the production of lower frequency signals (Bennet-Clark, 1998; Podos & Patek, 2015), a common pattern also found in other species of bushcrickets (Del Castillo & Gwynne, 2007). In these animals, frequency seems to be an honest signal for body size. Females discriminate for low-frequency song, which is associated with larger male body size (Latimer & Sippel, 1987; Gwynne & Bailey, 1988) and in *Poecilimon*, females consistently prefer heavier males based on their signal (Lehmann & Lehmann, 2008). Thus, larger sound generating structures might be a causal link between male quality and the signal produced. It would be extremely interesting to further investigate whether the size of sound generator organs can be considered a useful index for assessing signal honesty in Poecilimon species.

Males' acoustic activity is an honest predictor of both an individual's body condition and rival proximity.

Furthermore, in **Chapter 3**, the analysis of the signal behaviour in the bushcricket *P. ampliatus* showed that body mass affects male signalling activity, and this effect is amplified with the intensity of the contest. Signalling males apparently evaluate their rival's distance and manage the resources invested during acoustic performance. Bushcrickets signalling in aggregations have ample opportunities to gather information about other group members, and signalling males modify their activity depending on the presence and proximity of neighbouring rivals (Balakrishnan, 2016; Greenfield, 2015; Greenfield, Esquer-Garrigos, Streiff, & Party, 2016; Marin-Cudraz & Greenfield, 2016; Murphy, Thompson, & Schul, 2016).

Signal features appear to honestly convey information about the male's quality (Gray, 1997; Hack, 1997; Scheuber, Jacot, & Brinkhof, 2003; Shackleton, Jennions, & Hunt, 2005; Souroukis & Cade, 1993) and individuals reared under poor dietary conditions or infected by acoustically orienting flies decreased signalling activity (Lehmann & Lehmann, 2006). However, in many empirical studies it remains unclear whether results have really indicated the presence of additional costs for low-quality individuals for giving high-quality signals, when compared with high-quality individuals (Biernaskie, Grafen, & Perry, 2014; Higham, 2014).

In this study, the signal produced by males under strong competition pressure conveyed honest information about their body condition. In *P. ampliatus*, the low-quality, light males should have faced a higher cost for cheating or exhibiting a higher signalling activity than the high-quality, heavy males

did. However, when heavy and light males were tested in isolation or at the lowest competition pressure, their signals activity did not reflect information about their body condition nor about the cost paid to give their own signal. In contrast, when the light males were forced to exhibit an increased signal expression under the highest competition level, they did not increase their acoustic activity as the heavier males did. A possible explanation is the severe costs that light males are not able to pay to simulate a high-quality male's signal, under the maximum strength of competition. Thus, a more appropriate question might be:

Are there costs for light males giving a signal under the maximum competition pressure that heavy males do not face?

Even though the costs that light individuals paid to give their own signal (in terms of oxygen consumed or metabolic energies subtracted from other mating activities) were not directly measured in this study, it was partly shown, through testing individuals of different quality under different levels of competition pressure, how the stability and honesty of the acoustic signal might be ensured. It is possible to imagine a mutation that increases the signal performance of low-quality, light males within a population; however, the expected cost incurred by these "fake" signallers, in facing a stronger and closer rival, may explain why the honest acoustic signalling is maintained.

Male body mass and social context drive the inter-individual variation in signalling plasticity.

The findings in **Chapter 4** confirmed the decisive role played by the social context in determining the acoustic responses of competing males, presenting either similar or different body conditions. Acoustic contests seem to play an important role, in this species, in shaping the male's signalling strategy and plasticity when more than two competitors were signalling at the same time. As already found in *P. ampliatus* (**Chapter 3**), heavy focal males of *P. v. veluchianus* exhibited high plasticity in signalling behaviour, significantly varying their acoustic effort depending on the environment. However, contrary to what was expected, the strongest competition led heavy males to drastically decrease their signalling effort instead of increasing it. A perceived higher signalling pressure should cause an increase in male signalling performance, as shown in **Chapter 3** and in the previous study (Reichert & Gerhardt, 2012). It is likely that heavy males of *P. v. veluchianus* perceived the presence of the second rival in high body condition as an increase in the competition pressure, in terms of acoustic input perceived. Thus, it is possible that under the given level of competition, the focal male's signals might become too ineffective to be worth being answered. This might be particularly true in males of the *Poecilimon* species, where in nature rivals engage only in signal competition instead of physical fights (Lehmann, personal observations).

Since body quality is honestly advertised through specific features of acoustic signals (Gray, 1997; Hack, 1997; Scheuber, Jacot, & Brinkhof, 2003; Shackleton, Jennions, & Hunt, 2005; Souroukis & Cade, 1993), under competition focal males might be able to assess the body condition of the rivals by their acoustic signals. Indeed, heavy focal males change their signalling effort only when

competing against two rivals, but not against one. Moreover, heavy males put more effort into their signalling when competing against two light rivals in comparison to two heavy rivals. Being surrounded by close rivals of inferior body condition might increase their relative attractiveness. In fiddler crabs, it has been clearly shown that larger males are more likely to attract females if they display close to smaller rivals (Callander, Hayes, Jennions, & Backwell, 2012). So far, however, few other empirical studies have attempted to confirm whether competing males indeed benefit from associating with inferior rivals to increase their attractiveness and, hence, their mating success (Bateson & Healy, 2005; Oh & Badyaev, 2010).

Conversely, lighter males, in poorer body condition, and thus less competitive than heavier subjects, neither reduced nor stopped their signalling activity when the highest level of competition was tested (facing two heavy rivals). This lack of variation in light males' signals may lead to two possible conclusions. Firstly, it could reflect an alternative mating strategy that these individuals may adopt in nature: displaying near superior rivals, light males can increase their likelihood of mating with the females attracted by better-displaying males (i.e. "hotshot" models of lek formation, Beehler & Foster, 1988). Secondly, considering that behavioural plasticity itself is costly (DeWitt, Sih, & Wilson, 1998; Relyea, 2002), it could reflect the cost for light males to perform plastic behaviour: the trade-off between being an efficient signaller and coping with the costs of behavioural adjustments might be too high for low-quality males.

The evolutionary consequences of these findings may determine the presence in a population of several males' types which might increase their mating success, adopting different strategies depending on their body condition and environmental factors.

However, it is important to underline that, in both studies (**Chapter 3-4**), we used caged males. Thus, acoustic signalling is only one component so far considered so far between all possible displays that *Poecilimon* males could adopt for mate attraction.

5.3. Outlook

The present thesis deals with a critical question related to male honest acoustic signalling under sexual selection pressures:

Is the size of the signalling generator organs an honest cue of male quality, and how are these traits selected? How do internal and external factors affect the signing behaviour of individuals, given the existing energetic trade-offs? Is behavioural plasticity a means to cope with these costs?

To answer these questions both morphological and empirical evidence has been combined. The decisive role played by sexual selection in the size of structures adopted to generate acoustic traits preferred by females and in the plasticity of signal features during competition has been shown.

This thesis highlighted that male body mass alone is neither a necessary nor a sufficient feature of honest signalling. When individuals are forced to exhibit increased signal expression (to bear "out

of equilibrium" costs), the crucial role played by the social environment is enhanced. The competition level revealed a further constraint: for low-quality males, there is no net benefit cheating under the maximum competition strength.

The socially induced plasticity in sexually selected acoustic traits found in these two bushcricket species raises new hypotheses about the potential role that inter-sexual selection and natural selection could play in male choruses, where more than two competitors are signalling simultaneously. Consequently, new questions are opened up, such as: would males change their position, if they were free to move? Would light males try to intercept females approaching the high-quality signalling males?

It seems that under more complex environmental contexts, males select other strategies for mate signalling, and are likely to face higher energetic trade-offs. Thus, it is a priority for the future to test how different behavioural strategies, such as satellite or spacing behaviours, are selected during male contest and what is their link to sound performance. As found in other Orthoptera species (Arak & Eiriksson, 1992; Bailey & Field, 2000; Farris, Forrest, & Hoy, 1997; Forrest & Green, 1991), males may show either satellite or spacing behaviour to increase their chances of mating. In crickets, males tend to switch to silent searching at higher densities (Hissman, 1990; Simmons, 1986), with the frequency of satellite behaviour increasing with male density (Cade & Cade, 1992). Furthermore, Forrest & Green (1991) argued that quieter cricket males would gain the most advantage by spacing themselves outside the sound field of the louder males. Alternatively, Nityananda & Balakrishnan (2008), found that the quieter males in the choruses were spaced such that they were either entirely or partially within the sound field of the loudest male. This evidence suggests that these subjects may gain some advantage by being closer to the loudest male, perhaps because when a female is approaching, the signals' intensities are so high that she does not discriminate between the two.

Future research should therefore lead to more challenging empirical field studies, adopting individuals joining in bigger competitive interactions, to investigate how males alter multiple aspects of their mating behaviour under different levels of contest, connecting condition dependent signalling and alternative behavioural strategies.

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Eidesstattliche Erklärung

Hiermit erkläre ich, die vorliegende Dissertation selbstständig und ohne Verwendung unerlaubter Hilfe angefertigt zu haben. Die aus fremden Quellen direkt oder indirekt übernommenen Inhalte sind als solche kenntlich gemacht. Die Dissertation wird erstmalig und nur an der Humboldt-Universität zu Berlin eingereicht. Weiterhin erkläre ich, nicht bereits einen Doktortitel im Fach Biologie zu besitzen.

Marianna Anichini

Berlin, den

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Field of Slovenia. Foto of Marianna Anichini.